CANNABINOID conference 2011

IACM 6th Conference on Cannabinoids in Medicine and
5th European Workshop on Cannabinoid Research

September 8 – 10, 2011
University of Bonn, Germany

PROGRAM & ABSTRACTS

cannabinoid medicines International Association for Cannabinoid Medicines

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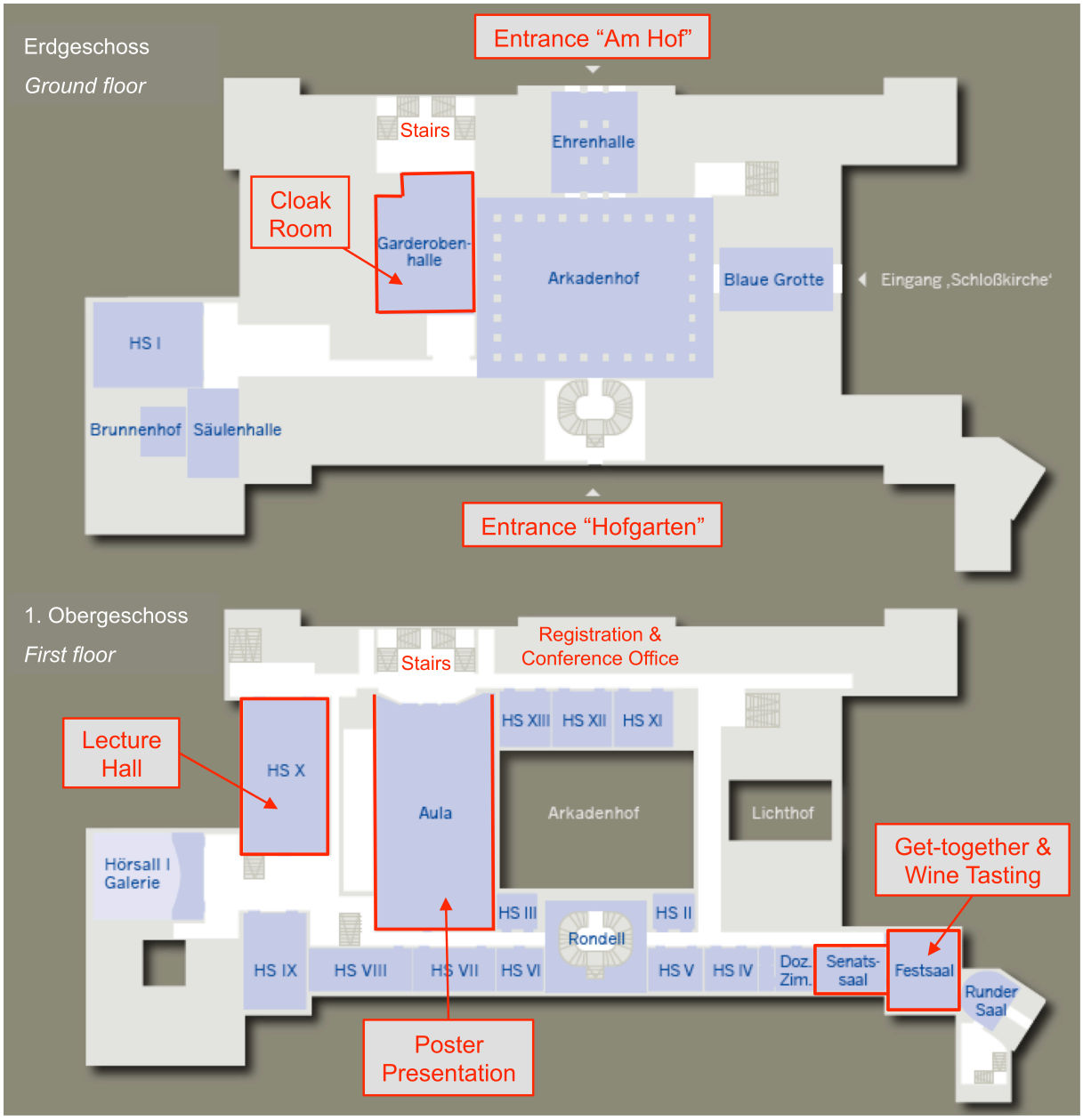
Floor plan



GENERAL INFORMATION

Badges

Please wear your badge at all times during the conference. You will also need to wear it for the conference dinner, in order to receive your drink coupons.

Conference Dinner

Dinner at Hotel Dreesen on Saturday, September 10, 7.30 p.m. to 10.00 p.m.

Rheinhotel Dreesen
Rheinstraße 45-49
53179 Bonn
Phone: +49 (0)228-8202-0
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www.rheinhoteldreesen.de

Hotel Dreesen is located in Bad Godesberg, some 8 km from Bonn City Centre. **We will provide bus service to/from the hotel**.

Busses will depart at **7.15 p.m.** from the “Am Hof” entrance of the University building.

The food as well as the first two drinks are covered by your registration fee. Please pay for any additional drinks. Coupons will be provided at the hotel, so please wear your badge for the dinner.

Conference Office

The Conference Office is located opposite the auditorium, beside Registration. The phone number is +49 (0)228 73-9486.

Poster Sessions

There will be two poster sessions at the conference:

Session 1, Friday, September 9, 15.00 - 17.00 p.m.
Session 2, Saturday, September 10, 13.15 - 14.30 p.m.

Both sessions will take place in the "Aula" (university auditorium). All posters will be available until the end of Poster Session 2 on Saturday. Presenters of posters with even numbers will be present at Poster Session 1, presenters of posters with odd numbers will be present at Poster Session 2.

Taxi

The nearest taxi rank is located at the central station, a 5-minute walk from the conference venue. To call a taxi, please dial +49 (0)228 55 55 55 or contact the Conference Office for assistance.

WLAN

WLAN access is provided in the lecture hall, the auditorium (“Aula”) and the surrounding hallways. Please pick up your user name and password at the Conference Office.

CONFERENCE PROGRAM

Thursday, September 8

13:00 - 19:00 **Registration**

15:00 - 15:15 Opening and Introduction

15:15 - 16:00 **Plenary Lecture I**
Daniele Piomelli
The Endocannabinoid System: New tools lead to new insights
Chair: A. Zimmer

16:00 - 16:30 COFFEE BREAK

16:30 - 18:15 **Session I – Brain functions**
Chair: M. Guzmán

16:30 - 17:00 **Ken Mackie**
Mechanisms of CB1 receptor desensitization

17:00 - 17:30 **Beat Lutz**
Cell type-specific functions of the brain's endocannabinoid system

17:30 - 17:45 **Ana Luisa Terzian**
The cross-talk between dopamine and cannabinoid in emotions and cognition: the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors

17:45 - 18:00 **Patrik Roser**
Auditory mismatch negativity generation in schizophrenia with and without comorbid cannabis use

18:00 - 18:15 **Miriam Schneider**
Social rejection in adolescent rats induces persistent alterations in pain perception and CB1 receptor expression - A new animal model with relevance for borderline personality disorder

18:30 - 21:00 GET-TOGETHER WITH WINE TASTING
Festsaal (see floor plan)

Friday, September 9

09:00 - 17:00 **Registration**

09:00 - 09:45 **Plenary Lecture II**
George Kunos
The peripheral endocannabinoid/CB1 receptor system as a novel therapeutic target in obesity/metabolic syndrome
Chair: I. Bab

09:45 - 10:00 COFFEE BREAK

10:00 - 11:45 **Session II – Metabolism and inflammation**
Chair: B. Lutz

10:00 - 10:30 **Vincenzo Di Marzo**
Targeting the peripheral endocannabinoid system with plant cannabinoids and n-3 polyunsaturated fatty acids to Combat abdominal obesity-associated metabolic disorders

10:30 - 11:00 **Sophie Lotersztajn**
Cannabinoid receptors in liver pathophysiology: new insights and therapeutic openings

11:00 - 11:15 **Zvi Vogel**
Effects of cannabinoids on microglial gene expression and their role in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis

11:15 - 11:30 **Sabine Steffens**
Deficiency of fatty acid amide hydrolase is associated with a vulnerable plaque phenotype in atherosclerosis-prone mice

11:30 - 11:45 **José Antonio Martínez Orgado**
Cannabidiol reduces hypoxic-ischemic brain damage by modulating excitoxicity, oxidative stress and inflammation. Role of CB2 and 5HT1A receptors

11:45 - 13:00 LUNCH

13:00 - 15:00 **Session III – Neuroinflammation**
Chair: S. Maione

13:00 - 13:30 **Javier Fernández-Ruiz**
Anti-inflammatory and neuroprotective potential of Sativex-like medicines in Huntington's disease: from basic research to clinical studies

13:30 - 14:00 **Andreas Zimmer**
CB2 receptor signaling in neuroinflammation

14:00 - 14:30 **Andrea Hohmann**
Role of endocannabinoid signalling in the control of pain initiation

14:30 - 14:45 **Onintza Sagredo**
Potential exidation of 2-AG by COX-2 enhances malonate toxicity in the striatum: Relevance for cannabinoid treatments in Huntington's Disease

14:45 - 15:00 **Judith Alferink**
CB2 signaling is required for susceptibility of cerebral malaria

15:00 - 17:00 POSTER SESSION I AND COFFEE BREAK
[IACM General Meeting]

17:00 - 18:30 **Session IV – Translational and preclinical studies**
Chair: J. Fernández-Ruiz

17:00 - 17:30 **Manuel Guzmán**
Therapeutic potential of cannabinoids as anticancer drugs

17:30 - 18:00 **Aron Lichtman**
Targeting Endocannabinoid Hydrolytic Enzymes to treat Neuropathic and Inflammatory Pain

18:00 - 18:15 **Evelyn Gaffal**
CB1 receptor deficiency in epidermal keratinocytes promotes contact allergic inflammation and delays epidermal barrier repair response

18:15 - 18:30 **Ethan Russo**
Cannabidiol and TRPV1: Turning down the heat (and pain)

Saturday, September 10

08:30 - 12:00 **Registration**

08:45 - 10:50 **Session V – Bone**
Chair: I. Bab

08:45 - 09:15 **Itai Bab**
Novel Cannabinoid Skeletal Targets

09:15 - 09.45 **Ruth Ross**
Cannabinoids and bone biology: latest developments

09:45 - 10:05 **Sabatino Maione**
The expression of TRPV1 channel and of CB1 vs CB2 cannabinoid receptors is modified in osteoclasts from osteoporotic women

10:05 - 10:20 **Thomas Randau**
CB2 receptor expression in human spinal mesenchymal stem cells from patients with osteoporosis

10:20 - 10:35 **Jürg Gertsch**
Osteoclastogenesis inhibition by a novel class of biphenyl-type cannabinoid CB2 receptor inverse agonists

10:35 - 10:50 COFFEE BREAK

11:05 - 12:05 **<u>Session VI - Herbal cannabis</u>**
Chair: W. Notcutt

10:50 - 11:20 **Roger Pertwee**
Phytocannabinoid Pharmacology: New Discoveries and Therapeutic Possibilities

11:20 - 11:35 **Jeffrey Hergenrather**
Clinical improvement and reduction of immunosuppressive drug therapy in cannabis treated patients with Crohn's Disease and ulcerative colitis

11:35 - 11:50 **Arno Hazekamp**
The medicinal use of cannabis and cannabinoids: an international survey on methods of intake

11:50 - 12:05 **Sarah Martin**
Cannabis oils produced by a multiple sclerosis patient reveals the potentials of cannabis when used in its natural form

12:05 - 13:15 LUNCH

13:15 - 14:30 POSTER SESSION II

14:30 - 16:30 **<u>Sativex, Nabilone, and Dronabinol</u>**
Chair: D. Abrams

14:30 - 15:00 **Philip Robson**
Sativex - a clinical overview

15:00 - 15:30 **William Notcutt –** Title N.N.
A Retrospective Description of the Use of Nabilone in UK Clinical Practice

15:30 - 16:00 **Tjalling Erkelens**
Bedrocan r&d –stimulating the development of herbal cannabis based –products

16:00 - 16:15 **Tim Beumer**
First in human trial of an oral tablet with Δ9-THC (Namisol®)

16:15 -16:30 **Franjo Grotenhermen**
Practical aspects of a treatment with dronabinol (THC)

16:30 - 17:00 BUSINESS MEETING
European Workshop on Cannabinoid Research

17:00 - 18:00 **Session VII – Hot topics from the lab and the clinic**
Chair: R. Pertwee

17:00 - 17:15 **Stefan Engeli**
Circulating endocannabinoids are related to blood pressure in patients with obstructive sleep apnea

17:15 - 17:30 **Attila Oláh**
Weed against ZIT? Cannabidiol inhibits lipid synthesis of human sebocytes both in vitro and in situ

17:30 - 17:45 **Linda Klumpers**
Demonstrating peripheral restriction of CB1 antagonist TM38837 in humans

17:45 - 18:00 **Viktor Rempel**
Xanthine derivatives as novel ligands for cannabinoid receptors and the related orphan receptor GPR55

18:00 - 18:30 **Special Presentation**
Raphael Mechoulam
Beyond Cannabis and Anandamide
Chair: A. Zimmer

19:30 - 22:00 CONFERENCE DINNER

ORAL PRESENTATION ABSTRACTS

THE ENDOCANNABINOID SYSTEM: NEW TOOLS LEAD TO NEW INSIGHTS

Daniele Piomelli, PhD.

Department of Pharmacology, University of California, Irvine, CA, USA
Drug Discovery and Development, Italian Institute of Technology, Italy

The endocannabinoids are a family of lipid messengers that engage the same cell surface receptors targeted by D^9-tetrahydrocannabinol, the active principle in marijuana. They are produced on demand through cleavage of membrane precursors and are employed in the execution of various short-range signaling processes. In the brain, they combine with CB_1 cannabinoid receptors on axon terminals to regulate ion channel activity and neurotransmitter release. Their ability to modulate synaptic efficacy has a surprisingly wide range of functional consequences and provides unique possibilities for therapeutic intervention. In my talk, I will describe the molecular and cellular mechanism responsible for producing and eliminating the endocannabinoids, anandamide and 2-arachidonoyl-*sn*-glycerol. I will focus in particular on the identification of a new intracellular anandamide-binding protein, called FAAH-Like Anandamide Transporter (FLAT), which facilitates the transmembrane transport of anandamide into neuronal cells. I will also outline the pharmacological properties and potential therapeutic applications of new chemicals that interfere with different aspects of endocannabinoid deactivation, including the FLAT inhibitor ARN272 and the peripherally restricted FAAH inhibitor URB937.

MECHANISMS OF CB_1 RECEPTOR DESENSITIZATION

Ken Mackie, Brian Davis, Alex Cook, Jill Farnsworth, David Marcus, Ramuh Shiva, and Dan Morgan[1]

[1]Department of Psychological and Brain Sciences and the Gill Center, University of Indiana, Bloomington, IN 47401, USA

Introduction: In humans, chronic use to cannabis leads to down regulation of CB1 receptors, while cessation of cannabis use in heavy users often causes a withdrawal syndrome. This process has been modeled in rodents, where repeated administration of cannabinoids leads to rapid tolerance, requiring more drug for the same effect. Using a cellular model of tolerance, desensitization of CB1 signaling, we found that phosphorylation of serines 426 and 430 in the CB1 receptor were necessary for rapid desensitization. To address the question if desensitization in cell systems and tolerance in an animal share a common mechanistic pathway, we made a knock in mouse where we mutated serines 426 and 430 to alanine, preventing their phosphorylation, and examined the response of these mice to cannabinoids.

Methods and Results: Conventional mouse genetic techniques were used to introduce targeted mutations in the CB1 gene. Initial characterization of the knockin mice revealed grossly normal cage behaviors, fertility and lifespan. Biochemical and anatomical characterization of the mice found an ~15% decrease in forebrain CB1 receptors, with a normal axonal distribution.

S426A/S430A knockin mice were more susceptible to the behavioral effects of THC, showing increased analgesia and hypothermia. This appeared to be due to enhanced sensitivity of CB1 signaling as a maximal dose (50 mg/kg) of THC produced similar degrees of analgesia and hypothermia in both wildtype and knockin mice. Knockin mice also developed tolerance more slowly than their wildtype littermates, with tolerance to the analgesic and hypothermic effects developing approximately three days later in the knockins. Precipitated withdrawal was enhanced in the knockin mice, demonstrating that these mice develop a higher degree of dependence than do wildtype mice. The knockin mice also showed enhanced sensitivity to the endocannabinoid, anandamide, suggesting that they might have enhanced endocannabinoid signaling.

Enhanced endocannabinoid signaling has been proposed to predispose individual to drug abuse. We examined if the S426A/S430A knockin mice were more sensitive to ethanol and more susceptible its rewarding effects. We found that to be the case, where the knockin mice consistently consumed more ethanol than did their wildtype littermates.

Conclusions: Our studies suggest that phosphorylation of serines 426 and 430 in the CB1 receptor are involved in limiting the acute response to THC as well as contributing to the development of rapid tolerance. However, phosphorylation of these residues is not the sole mediator of tolerance to THC, as tolerance slowly develops in the mutant mice. Furthermore, the S426A/S430A knockin mice appear to be a novel system for understanding the implications of "overactive endocannabinoid signaling".

CELL TYPE-SPECIFIC FUNCTIONS OF THE BRAIN'S ENDOCANNABINOID SYSTEM

Beat Lutz

Institute of Physiological Chemistry, University Medical Center Mainz, Duesbergweg 6, 55128 Mainz, Germany

The endocannabinoid system (ECS) constitutes a wide-spread signaling system in the organism, acting in a paracrine, autocrine and maybe even in an endocrine manner. Insights into the "logic" of the ECS have greatly progressed in the recent years, by extensively using pharmacological and genetic tools. The functional analysis of the CB1 receptor in the context of the entire animal has been the focus of our research. Using conditional mutagenesis in mice, cell-type and organ-specific CB1 receptor deletions have been generated and analyzed in a variety of physiological and pathophysiological processes. The recent establishment of a novel mutant CB1 receptor allele has enabled us to address the question on cell types and brain regions containing CB1 receptor where CB1 receptor expression is sufficient for a particular behavior. In addition, viral techniques using recombinant adeno-associated virus have been applied to overexpress various components of the ECS (e.g. CB1 receptor, fatty acid amide hydrolase, monoacyl glycerol lipase) in a cell-type and region-specific manner in the brain. The hippocampus and the amygdala have been of particular interest in the context of anxiety and fear learning behaviors.

THE CROSS-TALK BETWEEN DOPAMINE AND CANNABINOID IN EMOTIONS AND COGNITION: THE ROLE OF CANNABINOID CB1 RECEPTOR IN NEURONS EXPRESSING DOPAMINE D1 RECEPTORS

Ana Luisa Terzian[1,3], Fellipo Drago[2], Carsten T Wotjak[3], Vincenzo Micale[3]

[1]Graduate School of Systemic Neuroscience/Ludwig Maximilians Universität, Munich, Germany.
[2]Department of Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, University of Catania, Catania, Italy.
[3] Max-Planck Institute of Psychiatry, Munich, Germany

Although cannabinoid CB1 receptors are densely co-expressed with dopamine D1 receptors in the brain, little is known about their contribution to modulate emotional behaviors. We used conditional CB1 knock-out animals lacking CB1 receptors in neurons expressing D1 receptors (D1-CB1$^{-/-}$) in order to address this question. To elucidate the behavioral effects of CB1 deficiency in this specific neuronal subpopulation, we submitted D1-CB1$^{-/-}$ mice to a behavioral test battery, which included exploration-based tests, depressive-like behavioral tests, social behavior and fear-related memory paradigms. D1-CB1$^{-/-}$ did not show any difference in the exploration-based paradigms such as open field, elevated plus maze or object exploration test. Also, mutant mice performed normally in the forced swim test, a procedure widely used for evaluating behavioral despair in rodents. In contrast, they showed a mild anhedonia-like state as described by the weak decreased preference for sweet solution, as compared to control wild type (WT) group. This decrease, however, could be observed only during the first day of exposure, thus suggesting increased neophobia as an alternative explanation. However, weak- to moderate anxiety-like phenotypes were evident in D1-CB1$^{-/-}$ mice during social behaviors tests. Most strikingly, D1-CB1$^{-/-}$ mice exhibited a significant increase in contextual and auditory-cued fear, showing attenuation in within session extinction. This suggests that a specific reduction of endocannabinoid signaling in neurons expressing dopamine D1 receptors is able to affect acute fear adaptation. Our results provided first evidence for a cross-talk between dopaminergic and endocannabinoid systems in terms of controlling negative affects.

AUDITORY MISMATCH NEGATIVITY GENERATION IN SCHIZOPHRENIA WITH AND WITHOUT COMORBID CANNABIS USE

Patrik Roser, Ysabel Höpfner and Georg Juckel

Department of Psychiatry, LWL University Hospital, Ruhr-University Bochum, Germany

Introduction: Deficient mismatch negativity (MMN) generation as an indicator of auditory sensory memory and auditory information processing is a characteristic finding in patients with schizophrenia (SCH) and in individuals with cannabis use disorders (CUD). Given the hypothesized association between cannabis use and schizophrenia, this study investigated the effects of comorbid cannabis use in patients with schizophrenia.
Methods: Auditory MMN was assessed in 20 schizophrenic patients without cannabis use, 21 schizophrenic patients with cannabis use, and 20 healthy non-using controls. The MMNs to frequency and duration deviants were elicited within an auditory oddball paradigm and recorded by 32 channel EEG. Psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS).
Results: As expected, schizophrenic patients without cannabis use demonstrated reduced MMN amplitudes to duration deviants at frontal and central electrode positions compared to healthy controls. In contrast, schizophrenic patients with comorbid cannabis use showed greater MMN amplitudes to duration deviants at central electrodes compared to schizophrenic patients without cannabis use, whereas there were no significant differences compared to healthy controls.
Conclusions: These findings suggest that schizophrenic patients with comorbid cannabis use have superior cognitive functioning compared to non-using patients. It can be speculated whether these patients may represent a higher premorbid functioning subgroup of schizophrenia.

SOCIAL REJECTION IN ADOLESCENT RATS INDUCES PERSISTENT ALTERATIONS IN PAIN PERCEPTION AND CB1 RECEPTOR EXPRESSION – A NEW ANIMAL MODEL WITH RELEVANCE FOR BORDERLINE PERSONALITY DISORDER

Miriam Schneider[1], Christin Hannusch[1], Rainer Spanagel[2]

[1]Research Group Developmental Neuropsychopharmacology, Institute of Psychopharmacology, Central Institute of Mental Health (CIMH), University of Heidelberg, Germany; [2]Institute of Psychopharmacology, CIMH, University of Heidelberg, Germany

Introduction: Adolescence is a critical period for peer-rejection, since structural and functional maturation processes are taking place in brain regions underlying social cognition and social skills during this specific time of development. Disturbances in social interaction are characteristic for various mental disorders and are highly relevant for borderline personality disorder (BPD), which is characterized by a pervasive pattern of unstable interpersonal relationships and a hypersensitivity to rejection. Beside their constant fear of abandonment and rejection, adolescent BPD patients are indeed often excluded from peers, due to their behavior.

Methods: The aim of the present study was to establish an animal model for adolescent social exclusion and rejection. Therefore, female rats were subjected to different social housing conditions throughout adolescence. In the experimental group, rats of the playful Wistar strain (WIS) were paired with non-playful Fischer344 (FIS) rats. The inadequate pairing with a non-playful social partner in our model (WIS-FIS) reduces the occurrence of adequate playful activities for the Wistar rats, without depriving the animals of normal social contact. Pairs of female Wistar rats (WIS-WIS) served as controls.

Results: When tested as young adults – animals from the WIS-FIS housing conditions were found to express alterations in stress-reactivity and pain perception, compared to WIS-WIS controls. Additionally, enhanced CB1 receptor levels were detected exclusively in the thalamus of WIS-FIS rats compared to controls, indicating persistent alterations in endocannabinoid (ECB) signaling.

Conclusion: In conclusion, this novel animal model for adolescent social rejection leads to behavioral and molecular differences with relevance to BPD etiology and symptomatology. Since it is well known from human studies that social rejection induces similar neuronal reactions as physical pain, the present data indicate an important role for the ECB system in these processes.

TARGETING PERIPHERAL CB_1 RECEPTORS FOR THE TREATMENT OF VISCERAL OBESITY AND ITS METABOLIC COMPLICATIONS.

George Kunos and Joseph Tam
NIAAA/NIH, Bethesda, MD, USA

Obesity is associated with increased activity of the endocannabinoid system. Endocannabinoids are involved in regulating appetite, lipid metabolism and insulin sensitivity via CB_1 receptors located, at least in part, in peripheral tissues. CB_1 receptor inverse agonists are effective in reducing body weight and the associated metabolic complications, although adverse neuropsychiatric effects halted their therapeutic development. Such side effect may be minimized by selectively targeting peripheral CB_1 receptors. We have tested the metabolic and behavioral profile of the peripherally restricted CB_1 neutral antagonist AM6545 and the CB_1 inverse agonist JD-5037 as well as their brain-penetrant parent compounds in mouse models of obesity/metabolic syndrome. Both AM6545 and JD-5037 have <5% brain/plasma ratio and no significant CNS CB_1 occupancy after acute or chronic administration. Both compounds are devoid of behavioral effects mediated by central CB_1. In contrast, in mice with diet-induced obesity (DIO), both compounds reversed hepatic steatosis and dyslipidemias and improved glucose tolerance. AM6545 had minimal affects on food intake and body weight, whereas JD-5037 reduced food intake and body weight in DIO but not in ob/ob mice. The latter findings suggested leptin sensitization as the mechanism underlying the effects of JD-5037. Indeed, JD-treatment of DIO mice reversed their leptin resistance as indicated by the restored ability of leptin to reduce food intake, body weight, and STAT3 phosphorylation in the hypothalamus. JD-5037 treatment also rapidly reversed the hyperleptinemia of DIO mice by decreasing leptin secretion in adipose tissue and increasing leptin clearance via the kidney through a megalin-dependent mechanism. These findings indicate that inverse agonism at peripheral CB_1 receptors is a viable approach to the treatment of obesity and its metabolic complications while minimizing the chance of adverse neurobehavioral side effects.

TARGETING THE PERIPHERAL ENDOCANNABINOID SYSTEM WITH PLANT CANNABINOIDS AND N-3 POLYUNSATURATED FATTY ACIDS TO COMBAT ABDOMINAL OBESITY-ASSOCIATED METABOLIC DISORDERS

Vincenzo Di Marzo

Endocannabinoid Research Group, Institute of Biomolecular Chemistry, CNR, 80078 Pozzuoli (NA), Italy

Recent studies have shown that dysregulation of the endocannabinoid system in metabolically relevant peripheral tissues, such as the adipose tissue, liver and skeletal muscle, is responsible for part of the abdominal obesity-associated metabolic disorders, including insulin resistance, high triglycerides, low HDL-cholesterol and systemic inflammation, which are among the underlying causes of type-2 diabetes and atherosclerosis. On the other hand, agents that manipulate endocannabinoid tone in the brain might not be safe. Whilst the development of peripherally-restricted CB_1 antagonists is being actively pursued by some pharmaceutical companies, and targeting of CB_2 receptors for the treatment of fatty liver, pre-diabetes and atherosclerosis provided contradictory results, we are investigating the possibility, based on existing data in the literature or anecdotal reports, of employing less or non-psychotropic cannabinoids, such as tetrahydrocannabivarin (THCV) and cannabidiol (CBD), or dietary n-3 polyunsaturated fatty acids to combat metabolic disorders. In fact, THCV acts as a neutral CB_1 antagonist at low concentrations and inhibits food intake in lean mice, whereas CBD exhibits clear anti-inflammatory effects and reduces body weight in lean rats. Fish and krill oil are rich in esterified docosahexaenoic and eicosapentaenoic acids, which counteract inflammation and concomitantly reduce the levels of esterified arachidonic acid (AA) in phospholipids, that is, the ultimate precursor for endocannabinoid biosynthesis. Dietary n-3 polyunsaturated fatty acids appear to act mostly at the level of peripheral tissues in adult animals.
THCV and CBD enhance energy expenditure and reduced dyslipidemia in mice with diet-induced obesity (DIO) and *ob/ob* mice, respectively. To investigate the possible mechanisms for these effects, we used qRT-PCR, triglyceride staining with adipored® and various metabolomic approaches in isolated mouse adipocytes and myotubes and human hepatocytes treated with THCV or CBD. We found that these compounds reduce oleic acid-induced lipogenesis and/or enhance mitochondrial activity concomitantly to suppression of CB_1 mRNA expression. In Zucker rats and DIO mice, chronic (4-8 weeks) oral administration of krill oil (KO) reduced the amounts of AA-esterified to phospholipids in the liver and visceral adipose tissue or the levels of the direct endocannabinoid biosynthetic precursors, respectively. This was accompanied by reduced endocannabinoid levels in the liver/visceral adipose tissue and in the skeletal muscle, whilst concomitantly inhibiting ectopic fat accumulation and ameliorating insulin sensitivity, respectively. Dietary KO (12-24 weeks) reduced plasma anandamide levels and triglycerides also in obese men. In conclusion, targeting the peripheral endocannabinoid system with either plant cannabinoids or n-3 polyunsaturated fatty acids appears as promising strategies to counteract abdominal obesity-associated metabolic disorders.

Supported by research grants from GW Pharma and Aker Biomarine.

EFFECTS OF CANNABINOIDS ON MICROGLIAL GENE EXPRESSION AND THEIR ROLE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE), AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

Zvi Vogel[1,2], Ewa Kozela[1], Nirit Lev[3], Neta Rimmerman[1], Rivka Levy[2], Ana Juknat[1]

[1]Dr. Miriam and Sheldon G. Adelson Center for the Biology of Addictive Diseases, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978; [2]Neurobiology Department, Weizmann Institute of Science, Rehovot 76100; [3]Neurology Department, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Cannabinoids have been shown to exert anti-inflammatory activities including in experimental models of inflammatory CNS degenerative diseases. The use of cannabinoids, which interact with the neural cannabinoid receptor CB_1, is limited due to their psychotropic effects. Thus, cannabinoids which are deprived of psychoactivity, (*e.g.,* cannabidiol – CBD), are currently being tested for reducing neuroinflammation and neurodegeneration.

Using the BV-2 murine microglial cell line we found that CBD-treatment affected gene expression. The CBD gene expression profile showed changes associated with oxidative stress and glutathione depletion, involving the GCN2/eIF2α/p8/ATF4/CHOP-TRIB3 pathway. Furthermore, CBD stimulated genes were shown to be controlled by nuclear factors involved in the regulation of stress response and inflammation, mainly via the (EpRE/ARE)-Nrf2/ATF4 system and the Nrf2/Hmox axis. CBD was much more potent than the CB_1/CB_2 interacting Δ^9-tetrahydrocannabinol (THC), in inducing cellular stress response and anti-inflammatory activity (Juknat *et al*., Brit. J. Pharmacol. 2011). Moreover, at 10μM concentration, CBD, but much less so THC, increased BV-2 microglial cell death.

At the next step, we studied the effects of CBD, on myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. We observed that CBD administration during disease onset reduced EAE clinical symptoms. This was accompanied by diminished axonal damage and inflammation as well as reduced T cell recruitment in the spinal cord of the diseased mice. Treatment with CBD and several CBD derivatives also inhibited MOG-induced T cell proliferation *in vitro*. This later effect was not mediated via either the CB_1 or the CB_2 receptor and its target/receptor is not yet known (Kozela *et al*., Brit. J. Pharmacol. 2011). In summary, the non-psychoactive cannabinoid CBD has anti-inflammatory effects and reduces the clinical symptoms of MOG immunized EAE mice.

DEFICIENCY OF FATTY ACID AMIDE HYDROLASE IS ASSOCIATED WITH A VULNERABLE PLAQUE PHENOTYPE IN ATHEROSCLEROSIS-PRONE MICE

Sébastien Lenglet[1], Aurélien Thomas[2], Fabrizio Montecucco[1], Fabienne Burger[1], Graziano Pelli[1], Benjamin Cravatt[3], Christian Staub[2], Sabine Steffens[1]

[1]Division of Cardiology, University Hospital, Geneva, Switzerland, [2]Institute of Legal Medicine, Medical Faculty, University of Geneva, Switzerland, [3]The Scripps Research Institute, La Jolla, CA, USA

Recent evidence suggests an activation of the endocannabinoid system in the pathogenesis of atherosclerosis. However, evidence for a causal role of enhanced endocannabinoid signalling remains largely elusive. Here, we studied atherosclerosis development in mice lacking fatty acid amide hydrolase (FAAH), the enzyme responsible for endocannabinoid anandamide degradation.

Methods: We interbred FAAH-/- mice with apolipoprotein E (ApoE)-/- mice to generate ApoE-/-FAAH-/- mice. Before and after 5, 10 and 15 weeks on high cholesterol diet, we analyzed weight, serum cholesterol, atherosclerotic lesion size as well as plaque composition. Systemic levels of FAAH metabolites anandamide, palmitoyl- and oleoylethanolamide as well as endocannabinoid 2-arachidonoylglycerol (2-AG) which is metabolized by FAAH-independent pathways were also determined.

Results: Systemic levels of FAAH metabolites were about 2-fold higher in FAAH-deficient ApoE-/-mice. Conversely, serum levels of 2-AG were significantly increased in ApoE-/-, but not ApoE-/-FAAH-/- mice after 5 and 10 weeks of high cholesterol diet. After 15 weeks, we found less lipid content in aortic sinuses (1.4-fold smaller; $p<0.001$) and thoracic aortas (2.2-fold smaller; $p<0.01$) of ApoE-/-FAAH-/- mice in comparison to ApoE-/- controls. However, after 10 weeks on high cholesterol diet, plaques from ApoE-/-FAAH-/- mice had significantly increased neutrophil contents (ApoE-/-: 10.59±1.3; FAAH-/-ApoE-/-: 20.51±2.0), which correlated with increased MMP9 staining (r: 0.6529; $p<0.01$). No difference in plaque macrophage content was found. Conversely, the smooth muscle myosin-stained plaque area was significantly smaller in the double deficient mice (ApoE-/-: 33.04±1.4; FAAH-/-ApoE-/-: 24.70±2.3), suggesting a more vulnerable plaque phenotype.

Conclusions: Our data suggest that enhanced FAAH metabolite levels trigger the development of an unstable plaque phenotype and increased risk of plaque rupture. Thus, we may speculate that increased endocannabinoid anandamide levels found in patients with coronary artery disease might increase the risk for developing an acute clinical event due to plaque rupture.

CANNABIDIOL REDUCES HYPOXIC-ISCHEMIC BRAIN DAMAGE BY MODULATING EXCITOXICITY, OXIDATIVE STRESS AND INFLAMMATION. ROLE OF CB2 AND 5HT1A RECEPTORS.

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Background: cannabidiol (CBD) reduces hypoxic-ischemic (HI) brain damage in in vitro and in vivo models in newborn animals.
Aim: to characterize the mechanisms involved in CBD neuroprotection.
Methods: sedated and ventilated newborn pigs (1-2 day-old) underwent HI damage by adding hypoxia (FiO2 10%) to brain ischemia by bilateral carotid artery compression for 30 min. Half an hour after HI piglets received i.v. vehicle (HV, n=7) or CBD 1 mg/kg (HC, n=8), alone or with antagonists for CB2 (AM630, 1 mg/kg), 5HT1A (WAY100630, 0.1 mg/kg) or adenosine (caffeine, 10 mg/kg) receptors. Hemodynamic parameters and brain function (continuous amplitude-integrated EEG record) were monitorized up to six hours post-HI. At the end of experiment, piglets were euthanized and their brains removed; left brain hemisphere was frozen in isopentane and stored at -80ºC for biochemical studies (cytokine microarrays), whereas right hemisphere was preserved in 4% paraforlmadehide for histological studies (density of dead neurons by Nissl staininig). Samples from the frozen hemisphere were studied by proton magnetic spectroscopy (H-MRS) obtaining the following ratios: NAA/Cho (neuronal death), Lac/Cr (metabolic impairment), Glu/NAA (excitotoxicity), GSH/Cr (oxidative stress). Similarly studied animals without HI insult served as controls (SH, n=5)
Results: HI led to severe brain damage (26±3 vs. 5±1% dead neurons for HV and SH, $p<0.05$), which was associated with severe impairment of brain activity (final aEEG amplitude: 18±3% vs 85±7% baseline; aEEG trace clasification: 1.1±0.4 vs. 3.8±0.2 points, all for HV and SH, respectively, all $p<0.05$) and of H-MRS ratios, indicating increased cell death (reduced NAA/Cho: 4.7±0.5 vs. 7.1±0.6, for HV and SH, $p<0.05$) as well as metabolic derangement (increased Lac/Cr: 5.7±0.9 vs. 2.7±0.3, for HV and SH, $p<0.05$). CBD administration reduced histological brain damage (9±3% dead neurons, $p<0.05$ vs. HV), improved brain activity recovery (final aEEG amplitude: 65.5±9% baseline; aEEG trace 3.4±0.4 points, all $p<0.05$ vs HV) and normalized H-MRS ratios (NAA/Cho: 7.8±0.3, Lac/Cr: 3.4±0.3, both $p<0.05$ vs. HV). Such effects of CBD were related with the reduction of glutamate release (Glu/NAA ratio by H-MRS: 0.5±0.02, 0.62±0.03 y 0.5±0.03 for SH, HV y HC, $p<0.05$), oxidative stress (GSH/Cr ratio by H-MRS: 0.17±0.005, 0.11±0.01 y 0.17±0.01 for SH, HV y HC, $p<0.05$) and inflammation (IL-1: 116.7±7, 138±9 y 121±4 pg/mL; Il-6: 23±2, 28±3 y 22±2 pg/mL, for SH, HV y HC, $p<0.05$). CBD protective effects were abolished by coadministration of AM630 or WAY100630, but not by caffeine.
Conclusions: administration of CBD after a HI insult in newborn pigs reduces brain damage as assessed by histological, biochemical and metabolic studies, by acting on the more important mechanisms inducing brain damage as are excitotoxicity, oxidative stress and inflammation. CB2 and 5HT1A receptors are involved in CBD neuroprotection.

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ANTI-INFLAMMATORY AND NEUROPROTECTIVE POTENTIAL OF SATIVEX®-LIKE MEDICINES IN HUNTINGTON'S DISEASE: FROM BASIC RESEARCH TO CLINICAL STUDIES

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Several cannabinoid agonists afford neuroprotection in experimental models of Huntington's disease (HD), although the type of compound (i.e. CB_1 agonist, CB_2 agonist, antioxidant cannabinoid) most effective depends on the pathological characteristic(s) that mainly operate in each experimental model used. For example, CB_1 receptors become down-regulated, even in presymptomatic phases, in HD and their activation induces positive effects mainly in excitotoxic models (i.e. quinolinate-lesioned mice). CB_2 receptors, however, are up-regulated, mainly in glial elements, so that their pharmacological activation limits glial-derived events that aggravate striatal damage in animal models priming local inflammatory episodes (malonate-lesioned rats) and also excitotoxicity. In addition, antioxidant cannabinoids like cannabidiol (CBD) are effective against oxidant injury of striatal neurons as recapitulated in 3-nitropropionate-lesioned rats. Lastly, combinations of these effects have been found in the transgenic models that best reproduce HD pathogenesis (i.e. R6/2 mice). Therefore, these observations support the idea that the type of cannabinoid compound(s) that may be useful for a disease-modifying therapy in HD patients should be a multi-targeting cannabinoid or a combination of different selective compounds. We have proposed that the cannabis-based medicine Sativex®, which is a combination of botanical extracts enriched with either Δ^9-tetrahydrocannabinol (Δ^9-THC) or CBD, may serve this purpose in HD. Our proposal is based on evidence that a Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts attenuated cytotoxic events and preserved striatal neurons in the above models of HD in which striatal damage depends predominantly on a specific cytotoxic mechanisms. Indeed, this Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts removed the deficiency in endogenous antioxidant defenses and attenuated the up-regulation of calpain that occurs in 3-nitropropionate-lesioned rats. It reduced edema and normalized glutamate anomalies typical of quinolinate-lesioned mice, and it also reduced edema and inflammatory events (astrogliosis and microgliosis) predominantly associated with malonate toxicity in rats. The therapeutic potential of the combination of Δ^9-THC- and CBD-enriched botanical extracts is presently being studied in R6/2 mice and will soon be evaluated at the clinical level, in a trial directed at assessing the efficacy of Sativex® as a disease-modifying agent in a population of early symptomatic HD patients.

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CB2 RECEPTOR SIGNALING IN NEUROINFLAMMATION

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Modulation of inflammatory processes through the endocannabinoid system often involves cannabinoid CB2 receptor signaling, thus making this receptor an interesting pharmaceutical target. We have been particularly interested in the role of CB2 receptor signaling in chronic inflammatory and neuropathic pain conditions. Peripheral nerve injury typically leads to a long-lasting hyperalgesia and allodynia at the site of the injury Mice lacking CB2 receptors display a hyperalgesia response well beyond the local area normally affected by the nerve injury, accompanied by a wide-spread spinal microglia activation. Our findings suggest that CB2 activation attenuates the central inflammation after nerve injury through an IFN-γ dependent mechanism. Although endocannabinoids are most commonly viewed as signaling molecules that are produced on-demand, we found evidence that dietary phytocannabinoids may also contribute to the basal CB2 tone. Thus, the sesquiterpene beta-caryophyllene, a plant volatile found in large amounts in the essential oils of many different spice and food plants is a potent natural ligand of CB2 receptors. We demonstrate that beta-caryophyllene modulates neuropathic pain responses at physiologically relevant doses.

ROLE OF ENDOCANNABINOID SIGNALING IN THE CONTROL OF PAIN INITIATION

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Monoacylglycerol lipase (MGL) and fatty-acid amide hydrolase (FAAH) degrade the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), respectively. Selective inhibition of MGL and FAAH in the periphery may elucidate the role of endocannabinoid signaling in the control of pain initiation. We compared peripheral antinociceptive effects of JZL184, a selective MGL inhibitor, with URB602, an MGL-preferring inhibitor, and exogenous 2-AG to define the therapeutic potential of MGL inhibition outside the central nervous system (CNS) for suppressing inflammatory pain. Local administration of JZL184 inhibited MGL activity in rat paw skin without altering activity of enzymes implicated in degradation (FAAH) or synthesis (NAPE-PLD) of anandamide. URB602 also produced regionally-restricted increases in rat paw skin 2-AG levels, without altering levels of anandamide. Intra-paw administration of either JZL184 or URB602 suppressed both the early and late phases of formalin pain through CB_1 and CB_2-dependent mechanisms. Each MGL inhibitor also enhanced the antinociceptive effects of exogenous 2-AG. Inhibitors of MGL (JZL184) and FAAH (URB597), administered locally in the paw, also produced non-overlapping and modality specific suppressions of nociception produced by capsaicin, the pungent ingredient in hot chili peppers. We also compared effects of brain impermeant (URB937) and brain permeant (URB597) inhibitors of FAAH on nociception in models of inflammatory and neuropathic pain to better elucidate the role of anandamide signaling in the periphery in the control of pain initiation. URB937, a peripherally-restricted inhibitor of FAAH, suppressed both formalin-induced pain behavior and spinal Fos protein expression in a CB_1-dependent manner. Finally, we compared efficacy of a brain permeant (URB597), brain impermeant (URB937) inhibitors of FAAH with the brain permeant MGL inhibitor (JZL184) in a model of peripheral neuropathy produced by the chemotherapeutic agent cisplatin. Each endocannabinoid modulator suppressed cisplatin-evoked mechanical and cold allodynia by engaging CB_1 and CB_2-dependent mechanisms and normalized thresholds to pre-cisplatin levels. Our studies suggest that peripheral inhibition of FAAH outside the CNS is sufficient to suppress neuropathy produced by chemotherapeutic treatment. Our studies support the therapeutic potential of selective inhibition of FAAH and MGL outside the CNS for the treatment of inflammatory and neuropathic pain.

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POTENTIAL OXIDATION OF 2-AG BY COX-2 ENHANCES MALONATE TOXICITY IN THE STRIATUM: RELEVANCE FOR CANNABINOID TREATMENTS IN HUNTINGTON'S DISEASE

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Endocannabinoids, acting preferentially through CB_1 and/or CB_2 receptors, have demonstrated to serve as endogenous neuroprotective molecules against a variety of cytotoxic stimuli that operate in most neurodegenerative disorders including Huntington's disease (HD). However, emerging evidence indicate that endocannabinoids, in particular 2-arachidonoyl-glycerol (2-AG), may also exert cytotoxic effects as a consequence of their transformation in eicosanoid-related compounds by eicosanoid-related enzymes such as cyclooxygenase-2 (COX-2; see Kozak et al., 2004; Woodward et al., Pharmacol. Ther. 2008). We have also studied this possibility in an experimental model of HD, malonate-lesioned rats. For example, we reduced the levels of 2-AG by inhibition of diacylglycerol lipases (DAGLs) with O-3841 and found that, rather than enhancing malonate toxicity in the striatum, the reduction in 2-AG levels was accompanied by an attenuation of malonate-induced GABA and BDNF deficits, by an increase in the number of Nissl-stained cells, and by a reduction in the magnitude of malonate-induced astrogliosis (GFAP immunostaining). By contrast, the administration of OMDM169, an inhibitor of monoacylglycerol lipase (MAGL), which elevated 2-AG levels caused exactly the opposite effect. We hypothesized that both responses are related to 2-AG availability for COX-2-mediated biotransformation into prostaglandin glyceryl esters (PG-Gs). Indeed, we found that COX-2 is induced *in vivo* in the striatum 24 hours after the lesion, in parallel to equivalent responses in other proinflammatory enzymes like inducible NOS, and to opposite responses in anti-inflammatory mediators like nuclear receptors of the PPAR family. The increase in COX-2 was also reproduced *in vitro* in cultured M-213 cells exposed to malonate. *In vivo*, using a sensitive ESI-IT-ToF LC-MS technique, we could not detect the major 2-AG oxygenated metabolite, PGE2-Gs, presumably because the generation of this compound is strictly localised to the lesioned areas, thus reaching levels (<0.1 pmol/g tissue) that cannot be detected in the whole striatum with our method. However, levels of this metabolite (4.2 ± 1.0 pmol/mg lipid extract) could be detected in cultured M-213 cells after the addition of malonate and OMDM169, in parallel to an enhancement in cell death compared to cells exposed to malonate alone, as in the *in vivo* experiments. The addition of 2-AG *in vitro* also enhanced malonate effects, whereas the inhibition of DAGL with O-3841 produced the opposite effects. In summary, the availability of 2-AG for COX-2-mediated transformation into PG-Gs may represent a novel mechanism for this endocannabinoid to control neuronal survival that would add to its classic neuroprotective effects mediated by CB_1 and/or CB_2 receptors. We have studied this novel mechanism in an experimental model of HD.

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CB2 SIGNALUNG IS REQUIRED FOR SUSCEPTIBILITY OF CEREBRAL MALARIA

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It was suggested that the endocannabinoid system is functionally involved in controlling central nervous system (CNS) immune reactions. Cerebral malaria (CM) is a threatening complication of an infection with the parasite Plasmodium falciparum and may cause severe neurological deficits and mortality in infected individuals. Although previous work pointed to a neuroprotective effect of the cannabinoid receptor 2 (CB2) in CNS inflammation the functional role of CB2 in an antiparasitic immune response remains elusive up to now.

Methods: We used the infection of C57BL/6 mice with Plasmodium berghei ANKA (PbA) blood-stage parasites, which is a mouse model for CM leading to 100% mortality after 6 to 7 days of infection. In addition, a pharmacological approach was used by treatment of C57BL/6 mice with the CB2 antagonist SR122458.

Results: We found that *Cnr*2$^{-/-}$ mice were resistant to cerebral disease after this infectious challenge. A reduced blood vessel sequestration and an intact blood brain barrier identified in infected *Cnr*2$^{-/-}$ mice could cause increased resistance. In addition, diminished neuroinflammatory responses were found in *Cnr*2$^{-/-}$ animals with reduced numbers of CNS-immigrated CD8 T cells and activated myeloid cells. Importantly, therapeutic application of the CB2 antagonist SR122458 conferred enhanced CM resistance in C57BL/6 mice.

Conclusions: Our results clearly demonstrate an essential role for the endocannabinoid system in the pathogenesis of cerebral malaria with promising implications for CB2-based therapies in the treatment of antiparasitic responses of the CNS.

THERAPEUTIC POTENTIAL OF CANNABINOIDS AS ANTICANCER DRUGS

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Δ^9-Tetrahydrocannabinol and other cannabinoids inhibit cancer cell growth both in vitro and in various animal models. This anticancer activity is dependent on the modulation of key signalling pathways that trigger cell death as well as other events such as inhibition of tumour angiogenesis, cell proliferation and cell invasion. We have observed that THC induces glioma cell death through stimulation of autophagy and have unraveled the mechanism underlying this action by showing that THC, via ceramide accumulation, activates an endoplasmic reticulum stress response that promotes autophagy via inhibition of the Akt/mammalian target of rapamycin complex 1 (mTORC1) axis. We have also shown that autophagy is upstream of apoptosis in THC-induced cancer cell death and that activation of this pathway is necessary for THC anticancer action in mice. On the other hand, we have tested the combined effect of cannabinoids and different chemotherapeutic drugs, and have observed that administration of cannabinoids and temozolomide, the current benchmark for the management of glioma, synergistically reduces the growth of glioma xenografts in mice, an effect that seems to rely on the stimulation of autophagy-mediated cell death in those tumours. In addition, we have analysed the gene expression profile of a large series of human glioma cell lines, and have found a subset of genes with a marked differential expression in cannabinoid-sensitive vs. cannabinoid-resistant cells. Moreover, we have identified the growth factor midkine and its target the tyrosine kinase receptor ALK as crucial mediators of the resistance of glioma cells to cannabinoid anticancer action, thereby supporting the emerging notion that targeted inhibition of growth factor-evoked pro-survival signals can improve the efficacy of anticancer therapies. Altogether, these findings may set the basis for future clinical trials aimed at evaluating the potential activity of cannabinoids as anticancer agents.

TARGETING ENDOCANNABINOID HYDROLYTIC ENZYMES TO TREAT NEUROPATHIC AND INFLAMMATORY PAIN

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Methods The endogenous cannabinoids, N-arachidonoylethanolamide (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) are rapidly hydrolyzed by the respective enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Inhibition of these enzymes elevates endogenous cannabinoid levels and attenuates nociception in a variety of rodent models of pain. During this presentation, the consequences of acute and prolonged inhibition of FAAH and MAGL in neuropathic and inflammatory models of pain will discussed.

Methods: The FAAH inhibitor, PF-3845, and MAGL inhibitor, JZL184, were administered acutely or over the course of six days and evaluated in neuropathic (i.e., chronic constrictive injury of the sciatic nerve; CCI) and inflammatory (i.e., carrageenan) models of pain. Von Frey filaments were used to assess mechanical nociception. Complementary genetic (CB_1 (-/-) and CB_2 (-/-) mice) and pharmacological (i.e., rimonabant: CB_1 antagonist; SR144528 CB_2 antagonist) approaches were employed to evaluate the contribution of cannabinoid receptors.

Results: Acute administration of either endocannabinoid catabolic enzyme inhibitor reduced both neuropathic and inflammatory pain. Whereas the antinociceptive effects of high dose PF-3845 (10 mg/kg) were sustained upon repeated injections, prolonged administration of high dose JZL184 (40 mg/kg) led to tolerance in both pain models that was associated with CB_1 receptor down-regulation and desensitization, as well as cannabinoid dependence. Interestingly, low dose JZL184 (4 mg/kg) decreased neuropathic and inflammatory pain, these effects did not undergo tolerance after repeated administration, and there was no functional tolerance of the CB_1 receptor.

Conclusions: These results indicate that while FAAH inhibitors maintain efficacy in reducing neuropathic or inflammatory pain following chronic treatment, prolonged MAGL inhibition leads to functional tolerance of CB_1 receptor-mediated responses, accompanied with cannabinoid dependence. On the other hand, dialing down the dose of the MAGL inhibitor circumvents these problems associated with chronic blockade of this enzyme. Thus, both endocannabinoid catabolic enzymes represent viable targets to treat chronic pain conditions, though the degree to which they are inhibited is an important consideration.

CB1 RECEPTOR DEFICIENCY IN EPIDERMAL KERATINOCYTES PROMOTES CONTACT ALLERGIC INFLAMMATION AND DELAYS EPIDERMAL BARRIER REPAIR RESPONSE

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The mechanisms that regulate the magnitude and duration of allergic contact hypersensitivity (CHS) are incompletely understood. Here we further investigated how the endocannabinoid system attenuates CHS responses in mice. In experiments with cannabinoid (CB) receptor-deficient and wildtype mice involving adoptive lymphocyte transfer we found that endocannabinoids act predominantly on CB1 receptors expressed by radioresistant skin cells in the challenge phase of CHS. A time-course analysis of CHS responses in CB1 receptor-deficient mice ($Cnr1^{-/-}$) revealed enhanced and prolonged inflammation associated with hyperproliferation and abnormal differentiation of keratinocytes. Using a conditional gene-targeting approach, we observed that mice lacking CB1 receptors specifically in keratinocytes (KC-$Cnr1^{-/-}$) largely recapitulated the phenotype of complete $Cnr1^{-/-}$ mice, thus indicating that the essential CB1 receptor function resides in this cell type. Cultured CB1 receptor-deficient keratinocytes secreted lower levels of IL1α, a proinflammatory cytokine which supports the regeneration of injured epidermis. As an indicator of deficient IL1α-dependent defensive function *in vivo*, we discovered that both $Cnr1^{-/-}$ and KC-$Cnr1^{-/-}$ mice showed a delayed repair of the epidermal permeability barrier following challenges with contact allergen or acetone. Taken together, these results demonstrate a previously unrecognized pathophysiological role of CB1 receptors on keratinocytes, limiting contact allergic inflammation and promoting the homeostatic regeneration of epidermal integrity.

CANNABIDIOL AND TRPV1: TURNING DOWN THE HEAT (AND PAIN)

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Introduction: Great advances have been demonstrated in the last two decades in elucidating cannabinoid receptors, endocannabinoids and their metabolic enzymes, the components of the endocannabinoid system (ECS). The transient receptor potential (TRP) receptor family has also been identified during this same interval. It demonstrates considerable overlap with the ECS, inasmuch as anandamide (AEA) is not only an endogenous ligand for CB_1, but also for TRPV1, such that the latter may be considered an ionotropic cannabinoid receptor. Capsaicin is the classic agonist at TRPV1, and prolonged stimulation with it produces receptor desensitisation (De Petrocellis et al. *BJP* 2011, in press). Because this mechanism can be exploited to treat various chronic pain conditions and available TRPV1 antagonists have proven ineffective in clinical trials to date, it would be advantageous to identify non-noxious desensitising TRPV1 agonists for chronic therapeutic applications. Cannabidiol (CBD) appears to be one such candidate.
Methods: The current literature on various disorders was reviewed to form correlations between conditions mediated by TRPV1 and mechanisms of action of CBD.
Results: TRPV1 appears to be integral to the pathogenesis of numerous conditions, especially those involving neuropathic and visceral pain. Increased TRPV1 activity has been observed in both inflammatory and idiopathic bowel disease. Capsaicin has been noted to produce temporary exacerbation of symptoms, but reductions upon chronic administration. Analogous findings have been reported for idiopathic overactive bladder, as well as neurogenic detrusor overactivity associated with MS and spinal disorders. The high potency TRPV1 agonist/desensitiser, resiniferatoxin, has proven beneficial in treatment when administered to the bladder. Recent work has implicated TRPV1 as a mediator of endometrial pain, particularly in metastatic peritoneal involvement. Experiments in the rat demonstrated TRPV1 antagonism could reverse mechanical hyperalgesia and delayed onset muscle soreness, animal models for fibromyalgia (Fujii 2008). TRPV1 has been implicated in various migraine mechanisms, and while failure of two antagonists has been reported in human and animal experiments, clinical successes have been reported with repetitive applications of agonist/desensitisers including intranasal carbon dioxide, capsaicin, and civamide. Possible other applications extend to the realm of cutaneous pruritus, allergy, and even psychiatric conditions, such as anxiety. Cannabidiol has previously been reported to stimulate TRPV1, but also promote AEA release and inhibit its inactivation by fatty acid amidohydrolase (FAAH) (Bisogno et al. 2001). Recent results point to CBD as displaying 44.7% of efficacy of ionomycin at TRPV1, a potency at the receptor of 1.1 μM, and an IC_{50} of 0.6 μM for desensitisation as compared to capsaicin 0.1 μM (De Petrocellis et al. *BJP* 2011, in press).
Conclusion: CBD deserves clinical trial in numerous chronic pain conditions, whether administered systemically, or alternatively via focused applications such as intranasally to the sphenopalatine ganglion, topically, via trigger point injections, via rectal suppository, or intravesically through cystoscopy.

NOVEL CANNABINOID SKELETAL TARGETS

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Skeletal strength primarily depends on bone density and architecture, which are in turn determined by bone remodeling, an ongoing renewal process consisting of bone tissue resorption by osteoclasts and new bone formation by osteoblasts. It is now established that the endocannabinoid (EC) system is an important regulator of bone remodeling and bone mass, through the activation of CB1 receptors expressed in skeletal sympathetic nerve terminals and CB2 receptors expressed in osteoblasts and osteoclast. We now show evidence for that CB1 is a link in a 2-AG – norepinephrine (NE) feedback circuit, whereby NE, which inhibits osteoblastic activity, stimulates 2-AG synthesis in these cells. The elevated extracellular 2-AG increases sympathetic CB1 activity, which induces a decrease in skeletal NE levels, thus alleviating the negative adrenergic tone of bone formation.

Another critical skeletal activity is driving body growth. In vertebrates, skeletal elongation occurs mainly by enchondral ossification, an intricate process controlled by multiple hormones and growth factors. Cannabis use during pregnancy leads to babies shorter than those born to nonusers, suggesting the occurrence of an EC system intrinsic to the epiphyseal growth plate (EGP), which drives skeletal elongation. Indeed, the EGP harbors a cannabinoid system consisting of CB1 and CB2 receptors specifically expressed in hypertrophic chondrocytes. These cells also express diacylglycerol lipase (DAGL) α and DAGLβ. CB1 and/or CB2 deficient mice at the end of the accelerated growth phase are longer than wild type controls. THC administration to normal mice during their rapid growth phase (5-11 weeks of age) slows down skeletal growth. *Ex vivo* THC challenging of growth plate chondrocytes inhibites hypertrophic-cell nodule formation. These findings demonstrate a local EC system in the EGP with a growth inhibitory role for CB1 and CB2, suggesting a physiologic role for the EC system in the regulation of growth cessation.

THE EXPRESSION OF TRPV1 CHANNEL AND OF CB1 *VS* CB2 CANNABINOID RECEPTORS IS MODIFIED IN OSTEOCLASTS FROM OSTEOPOROTIC WOMEN

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Human osteoclasts in vitro and ex-vivo express functional TRPV1 channels, CB1/CB2 cannabinoid receptors and endocannabinoid/endovanilloid synthetic/catabolic enzymes. Pharmacologic manipulation of this system can modulate osteoclast activity. Here, through multidisciplinary approaches, we looked for possible changes in endocannabinoid/vanilloid system during menopause as compared to primary osteoporosis, demonstrating that endocannabinoid/endovanilloid system enzymes and receptors are differently expressed in osteoclasts from menopausal women without or with osteoporosis. We observed that in osteoclasts from osteoporotic patients TRPV1 channels are up-regulated and, if persistently stimulated with resiniferatoxin, become clustered to the plasma membrane whilst inducing a massive CB2 receptor over-expression. By providing new evidence for a critical functional cross-talk between CB2 and TRPV1 receptors in osteoporosis, we speculate that TRPV1 desensitization, or its enhanced trafficking, together with TRPV1 agonist-induced CB2 receptor over-expression, might be critical for avoiding massive calcium entry in osteoclasts which could be responsible of cell over-activation and higher bone resorption or excitotoxicity.

CB2 RECEPTOR EXPRESSION IN HUMAN SPINAL MESENCHYMAL STEM CELLS FROM PATIENTS WITH OSTEOPOROSIS

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Background: Osteoporosis is recognized among the 10 most crucial global diseases, increasing the medical and socioeconomic burden steadily in the near future. It is defined as a systemic deterioration of bone mass and micro-architecture, disturbing the fragile coupling of bone-forming (osteoblasts) and bone-resorbing cells (osteoclasts). The spine is mostly commonly affected, and subsequently vertebral compression fractures are the most frequent osteoporotic fractures. Bone marrow, among other niches, harbours mesenchymal stem cells (MSCs), the progenitors of different mesodermal cell types. Whether dysfunction or deficiency of MSCs contributes to the pathogenesis of osteoporosis is still unclear, and their potential for cell based therapy remains a challenge. The cannabinoid receptor 2 (CBR2) was found to be expressed by osteoclasts and osteoblasts, playing a role in keeping bone remodelling at balance. It has been reported that CBR2 polymorphisms were clearly associated with postmenopausal osteoporosis, thus CBR2 represents a molecular target for osteoporosis treatment. The aims of this project is therefore to prove that fractured vertebral bodies harbour a population of MSCs, and how CBR2 expression differs from healthy donors in these MSCs.

Methods: Bone marrow aspirate and bone biopsies were taken from osteoporotic and healthy donors undergoing spinal surgery. MSCs were isolated, expanded and characterized through differentiation, immunomodulatory capacity and typical stem cell marker expression. CBR2 expression was investigated via immnuhistochemistry, both in bone tissue biopsies as well as in undifferentiated MSCs. CBR2 expression was analysed quantitatively using FACS technology.

Results: The isolated cells were plastic adherent, proliferating, and showed a typical phenotype of MSCs. FACS analysis showed typical stem-cell like expression pattern of CD45 (neg), CD105 (pos) and CD166 (pos). Plated cells were also able to effectively suppress the proliferation of activated lymphocytes in an immunoassay. MSCs were successfully differentiated toward osteogenic, adipogenic and chondrogenic lineages which were assessed via corresponding stainings. Healthy and osteoporotic donors expressed CBR2 differently, both in bone biopsies and MSCs: CBR2 was strongly manifested in the MSCs of osteoporotic donors, whereas nearly absent in healthy donors. The difference was not maintained in adult osteocytes in bone biopsies.

Conclusions: In summary**,** we were able to isolate, expand and characterize MSCs from vertebral bodies with osteoporotic compression fractures. These MSCs possess a similar phenotype and have the same chacteristics, surface markers and immunomodularory capacity as MSCs of healthy donors. Furtheremore, strong CBR2 expression was detected in MSCs of osteoporotic donors, which was not seen in MSCs of healthy patients. This suggests a differential regulation of the CBR2 pathway in these early progenitor cells, underlying the potential dysfunction of the differentiated osteoblasts and the subsequent bone loss. In the bone biopsies, however, the osteocytes in osteoporotic tissue did not show a higher expression as compared to healthy biopises, indicating that the role of CBR2 regulation in osteoporosis might be set in the early stage of osteoblast differentiation

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OSTEOCLASTOGENESIS INHIBITION BY A NOVEL CLASS OF BIPHENYL-TYPE CANNABINOID CB_2 RECEPTOR INVERSE AGONISTS

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The cannabinoid CB_2 receptor is known to modulate osteoclast function by poorly understood mechanisms. We report that the natural biphenyl neolignan 4'-*O*-methylhonokiol (MH) from *Magnolia grandiflora* is a CB2 receptor-selective anti-osteoclastogenic lead structure (K_i < 50 nM) and counteracts 2-AG at the level of cAMP (Schuhly et al. Chemistry & Biology 18, 1–12, 2011).

Methods: Bioactivity-guided isolation led to the identification of a new CB2 receptor scaffold, which was extensively profiled on an array of receptors. Chemical derivatization of 4'-*O*-methylhonokiol resulted in a library of compounds that were subjected to CB2 receptor binding and functional assays. The most active compounds were tested on osteoclastogenesis assays, migration assays and LPS-stimulated TNF-alpha expression, using primary human CD14+ precursor cells.

Results: Intriguingly, MH and several derivatives triggered a simultaneous G_i inverse agonist response and a strong CB_2 receptor-dependent increase in intracellular calcium via the CP55,940 competitive binding site, indicating unexpected plasticity of CB2 receptor signalling. The most active inverse agonists from a library of MH derivatives inhibited osteoclastogenesis in RANK ligand-stimulated primary human macrophages. Moreover, these ligands potently inhibited the osteoclastogenic action of endocannabinoids. Our data show that CB2 receptor-mediated cAMP formation, but not intracellular calcium, is crucially involved in the regulation of osteoclastogenesis, primarily by inhibiting macrophage chemotaxis and TNF-alpha expression.

Conclusions: We describe a novel type of biphenyl CB2 receptor-selective ligand that exerts a unique mixed functional effect. Our data indicate that nM concentrations of 2-AG increase osteoclast formation via activation of migration, thus facilitating syncytium formation. Moreover, in our assays with M-CSF/RANKL stimulated primary human CD14+ cells CB2 receptor inverse agonists inhibit osteoclastogenesis and intracellular calcium transients do not play a role. MH is an easily accessible CB2 receptor-selective scaffold that exhibits a novel type of functional heterogeneity and shows intriguing similarities to previously described *Cannabis*-derived biphenyls.

PHYTOCANNABINOID PHARMACOLOGY: NEW DISCOVERIES AND THERAPEUTIC POSSIBILITIES

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Evidence continues to emerge that cannabis is the source of several compounds in addition to Δ^9-tetrahydrocannabinol that could be used as medicines by themselves or in combination with other phytocannabinoids or with non-cannabinoids. Some of this evidence has come from recent research into the pharmacological actions of Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidiol (CBD), cannabigerol (CBG) and omega-3 fatty acids that we have performed. Thus, we have discovered that Δ^9-THCV is both a CB_2 receptor agonist and a CB_1 receptor antagonist, a combination of pharmacological actions that could possibly be exploited in the clinic for the management of disorders such as inflammation and inflammatory pain, Parkinson's disease, stroke and liver damage. Indeed, evidence has very recently emerged from animal experiments that Δ^9-THCV would be effective against all these disorders. That Δ^9-THCV would also be effective against systemic sclerosis, nicotine dependence/relapse, obesity, epilepsy and osteoporosis is likely too, as is CBD-induced enhancement of the probable ability of Δ^9-THCV to ameliorate Parkinson's disease. These possibilities merit additional investigation as does further exploration of the pharmacological actions of Δ^9-THCV. Turning now to CBD, it is becoming increasingly likely that several potential therapeutic effects of this phytocannabinoid depend on increased activation of 5-HT_{1A} receptors. Thus, the ability of CBD to prevent cerebral infarction, to reduce signs of anxiety and depression, to ameliorate cognitive and motor impairments and to decrease vomiting and signs of nausea in animals all seem to result from increased 5-HT_{1A} receptor activation. We are currently seeking out the mechanism(s) by which CBD induces such activation since, although there is already evidence from *in vitro* experiments that CBD can activate 5-HT_{1A} receptors directly, this agonism was only observed at the rather high CBD concentration of 16 μM. As to CBG, we have discovered that, in contrast to CBD, it can potently block 5-HT_{1A} receptors. It will be of interest, therefore, to establish whether CBG is effective against negative symptoms and impaired cognition of schizophrenia and, indeed, against depression or neuropathic pain. That CBG may have analgesic properties is also supported by our finding that it behaves as a potent α_2-adrenoceptor agonist. Finally, there is evidence that two omega-3 fatty acids that are present in fish oil, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), can enhance the apoptotic efficacy of docetaxel in prostate cancer cells. When ingested, DHA and EPA are converted to ethanolamides which we have found recently to induce anti-proliferative effects in prostate cancer cells more potently than their parent compounds. These ethanolamides should probably be classified as endocannabinoids, since they can activate cannabinoid CB_1 and CB_2 receptors. Hemp seed oil is the source of an omega-3 fatty acid, α-linolenic acid (ALA), and we are currently exploring the ability of its ethanolamide to interact with CB_1 and CB_2 receptors. Further experiments are also now underway to investigate the impact of fish oil and hemp seed oil omega-3 fatty acids on the endocannabinoid system. Our latest findings with Δ^9-THCV, CBD, CBG and omega-3 fatty acid ethanolamides will be presented.

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CLINICAL IMPROVEMENT AND REDUCTION OF IMMUNOSUPPRESSIVE DRUG THERAPY IN CANNABIS TREATED PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS

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California physicians involved in the practice of cannabis consultations regularly encounter patients with autoimmune and idiopathic inflammatory conditions; a large proportion of these have inflammatory bowel disease. SCC physicians performed a retrospective evaluation of 38 patients with an independent diagnosis of Crohn's disease (ICD-9 555.9) and ulcerative colitis (ICD-9 556.9). The primary aim of the study was to evaluate the efficacy of ad lib use of cannabis in alleviating symptoms of active inflammatory bowel disease, both with and without concomitant use of conventional medications. A secondary aim was to determine if cannabis alone or in combination with conventional medications leads to better response, longer periods of disease quiescence, and reduction in the use of conventional medications. Conventional medications included immunosuppressive drugs, anti-inflammatory drugs, steroids, antimicrobials, and TNF-alpha binding medications.

All study patients were self-referred and approved to use cannabis for relief of the symptoms of inflammatory bowel disease. Study inclusion criteria included the completion of a questionnaire designed to elicit details of the clinical course and use of all medications, including cannabis. The patients utilized their own supply of cannabis with methods of administration of their choice. The quantity of cannabis used was self-adjusted in accordance with symptoms, concomitant use of other medications, side effects, and employment considerations.

Patients report statistically significant improvement in their signs and symptoms when using cannabis. Stools per day, flare-up frequency, and flare-up severity were significantly reduced when patients used cannabis (all $p < .001$). Patients' appetite, activity, and average weight all increased significantly with the use of cannabis (all $p < .01$). A scale of symptoms including pain, nausea, vomiting, fatigue, and depression showed an average reduction by almost 50% when patients used cannabis (mean score using cannabis = 12.87, mean score without cannabis = 25.67, $p < .001$). Patients report a marked reduction of conventional pharmacotherapy associated with the regular use of cannabis.

Cannabis is preferred over conventional medications with nearly half of the study patients using cannabis only in their daily management of inflammatory bowel disease. Cannabis is believed to function as an effective immunomodulator, appetite stimulant, antispasmodic, and pain relieving medication with a wide margin of safety.

THE MEDICINAL USE OF CANNABIS AND CANNABINOIDS: AN INTERNATIONAL SURVEY ON METHODS OF INTAKE

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Introduction: Only limited information is available on advantages and disadvantages of different methods of intake (oral, oro-mucosal, inhalation) of cannabis products and different cannabinoid-based medicines as well as preferences by patients. Few clinical studies have directly compared the effects of these medicines on patients (Abrams et al., Ann. Intern. Med. 2003;139(4):258-266; Zajicek et al., J. Neurol. Neurosurg. Psychiatry 2005;76(12):1664-1669) or healthy subjects (Hart et al., Psychopharmacology (Berl) 164(4):407-415; Wachtel et al., Psychopharmacology (Berl) 161(4):331-339). Therefore, a questionnaire was designed to determine how patients perceive possible advantages and disadvantages of different methods of intake and which methods or products they prefer over others. The study also intended to analyze whether perceived advantages and preferences depend on demographic parameters (gender, age and country), previous experience with recreational cannabis use, disease and/or symptoms, or involvement of a physician in the use of cannabinoids.
Methods: A cross-sectional survey was conducted by putting a questionnaire on the website of the IACM (International Association for Cannabinod Medicines, http://www.cannabis-med.org) between 18 August 2009 and 31 January 2010. It was available in five languages (German, English, Spanish, French, Dutch). It consisted of 22 questions, each of which could be answered by binary (yes/no) response, a list from which to choose most-suited answers, or likert scale. Two open-ended questions were included to allow free comments. The collected information included demographics, diseases and symptoms, medical treatment, cannabis use pattern, dose, onset of effects and methods of former and current intake of cannabis or cannabinoids. Participants were asked on advantages of different methods of intake, including onset of effects, ease of dose finding, side-effects, amount of cannabis needed, etc.
Results: 953 patients (614 male, 339 female) with a mean age of 40.7 years from 32 countries completed the questionnaire. Most participants were from the USA, Germany, France, Canada, The Netherlands, Spain, and the UK. In 47.6% of all cases, cannabis products were prescribed or recommended by a physician, in 10.4% patients got their cannabinoid medication from a pharmacy, in 26.3% from a coffee shop or another unofficial or tolerated source. In 54.4% of cases the cannabis products were (also) home grown (legal or illegal). The highest percentage of prescribed or recommended cannabinoid medication was found in the USA, The Netherlands, Canada, and Germany. 76.5% of participants had experience with cannabis products before the onset of disease. Preferred modes of use were smoking of cannabis (62.9%), inhalation of cannabis with a vaporizer (23.6%), oral use of cannabis in baked goods (7.9%), oral use of cannabis as a tea (2.4%), and oral use of dronabinol/Marinol (1.8%). No significant differences in preferred modes of use were found in correlation to symptoms or diseases. Further results will be presented.
Conclusions: The IACM survey provides the largest database of information so far on patients' preferences with regard to the medical use of cannabinoids in correlation to a large number of variables (disease, symptoms, demographic parameters, etc.).

CANNABIS OILS PRODUCED BY A MULTIPLE SCLEROSIS PATIENT REVEALS THE POTENTIALS OF CANNABIS WHEN USED IN ITS NATURAL FORM

Sarah Martin

A British Multiple Sclerosis patient shares her research of cannabinoids and how she successfully produces her own cannabis medicines. With no legal access to medical grade cannabis flowers, she was also denied access to Sativex, a legal cannabis based drug. She realises how cannabinoids will lead her to relief from crippling symptoms. First Inhaling cannabis using a vaporiser, she was led on to extract cannabis oil to produce effective tinctures to treat symptoms of her disease. This presentation reveals real world techniques for simple cultivation and synthesis of cannabis medicines suitable for for people with MS and a variety of chronic illnesses. Sarah has been in serious relapse for much of 2011 and wishes to share anecdotes of her recovery as she medicates using cannabis oils.

Methods: Three strains of cannabis, ranging from Saliva to Indica varieties, will be processed into oil using Butane extraction. The effectiveness of each strain will be assessed on how it reduces symptoms and enhance day to day living.

SATIVEX: A CLINICAL OVERVIEW

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Sativex is a standardised cannabinoid medicine derived from whole-plant extracts of two chemotypes of *Cannabis sativa L.* prescribed in the form of an oro-mucosal spray. Each actuation delivers 2.7mg delta-9-tetrahydrocannabinol (THC) and 2.5mg cannabidiol (CBD) along with defined quantities of minor cannabinoids and terpenoids, and the excipients ethanol, propylene glycol and peppermint oil.

The first exploratory clinical trials of Sativex began in 2000 in Great Yarmouth and Oxford. Subsequently, more than 1400 patient-years of treatment in clinical trials have been accumulated in 22 countries in recipients with ages ranging from 19 – 78 years. In addition to this, there have been more than 8000 patient-years of monitored prescription.

To be eligible for enrolment in Sativex clinical trials all patients must have failed to respond adequately to standard treatments, and stable concomitant medications are maintained throughout. Individual doses of Sativex (and placebo) are established by an initial period of self-titration within clear guidelines, and the average maintenance dose in clinical trials has been approximately 8 sprays / 24h. There has been no evidence of tolerance on long-term dosing, and no reports of misuse have been received to date.

Sativex is currently licenced and commercially available in Canada, UK, Spain, Germany and Denmark, and launches are anticipated soon in Italy, Sweden, Austria, and the Czech Republic. The primary indication is as an add-on treatment for spasticity in multiple sclerosis (MS) in patients who have not responded adequately to other anti-spasticity medication. It is also under investigation for the treatment of various types of chronic pain.

Evidence for the efficacy and safety of Sativex in a pivotal clinical trial of MS-related spasticity, and in Phase II trials of neuropathic, inflammatory and cancer pain will be summarised. Plans for an international Phase III programme in cancer pain will be outlined.

A RETROSPECTIVE DESCRIPTION OF THE USE OF NABILONE IN UK CLINICAL PRACTICE

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Introduction

Interest in the clinical applications of endocannabinoid therapy has been growing. From 1985 to 2010 Nabilone has been the only medicinal cannabinoid available in the UK, licenced for the treatment of chemotherapy-induced nausea and vomiting. Since the early 1990s there has been increasing off-label use for the treatment of chronic pain and spasticity. An observational study to describe patterns of current clinical use in the UK was undertaken.

Methods

Ethics approval from Cambridge was obtained. 3 hospitals were identified by MEDA Pharmaceuticals as high users of Nabilone. Patients for whom Nabilone had been prescribed in the last 5 years were identified from pharmacy records. A questionnaire on Nabilone use was designed and completed using the clinical notes by members of the prescribers' teams. Data management was by pH Associates.

Results

Records of 134 patients from 3 centres were studied, including a sub-population of 38 MS patients from 2 centres. Overall 59% were female as were 66% of the MS group. The mean age of diagnosis was 41 years but that of first Nabilone use at 49 (52 for the MS sub-group). The mean duration of symptoms varied between 6 years at one centre to 12 years at another. The range of non MS diagnoses was very wide. Pain (87%) and Spasticity/spasms (25%) were the commonest symptoms treated. In only 3 patients was Nabilone used principally as an anti-emetic.

A review of previous treatment showed the expected wide range of medications, recognising that Nabilone was only used when all else had failed (including several others that were used off-label eg. Ketamine for pain21.6%; Anticonvulsants in MS 59%).

The commonest starting dose at 2 centres was 1mg. The other centre normally started lower at 0.25 or 0.5mgs, either with 0.25mg capsules or with capsule splitting. For 91%, Nabilone was started at night. The final titrated dose was 1mg for 65% of patients with a range of 0.25mg to 6mg. This was similar for the MS sub-group. The outcome of Nabilone use was only documented in 72% of patients. The major benefits were improvements in pain 78%, spasticity 19%, and sleep 49%. The results for the MS sub-group were similar. 16% of patients suffered 1 side-effects and 7% 2 or more. Drowsiness was by far the most common but only 1 suffered hallucinations and none dysphoria.

Overall 41% of non-MS patients and 70% in the MS group discontinued Nabilone. 56% had done so within 3 months although the data was only available for 27 of 65 patients. The main reasons were lack of benefit 56% and side-effects 29%. A starting dose of 1-2mg was associated with a much higher level of discontinuation although this didn't reflect a substantially higher level of side effects. 30% have been using Nabilone for >2 years.

Discussion

Lack of use of assessment tools for benefit and for side-effects was a major deficiency requiring improvement. However, the ongoing benefit and limited side effects were reassuring.

BEDROCAN R&D –STIMULATING THE DEVELOPMENT OF HERBAL CANNABIS BASED –PRODUCTS

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Medicinal grade cannabis has been available by prescription in The Netherlands since 2003. Bedrocan BV is currently the only grower contracted by the Dutch Ministry of Health to produce cannabis for this national program. The Office for Medicinal Cannabis (OMC) oversees the program and ensures a sufficient supply for patients, research, and the development of cannabis-based medicine.

The successful medicinal use of cannabis depends on more than the availability of herbal material alone. A thorough understanding of chemical composition, proper dosing methods, and efficient modes of administration are crucial to turn a much-debated treatment into a medicine by modern standards. Clinical trials may then be able to show when and where herbal cannabis can be considered as proper treatment.

With 4 different varieties of cannabis currently available to Dutch patients, Bedrocan BV has shown its ability to standardize production according to pharmaceutical standards, and to incorporate patients' needs into its cultivation program. Worldwide, the Netherlands is the only country offering patients a choice of medicinal varieties, including sativa- and indica-types of cannabis, as well as a product containing both THC and CBD. The medicinal cannabis program has been steadily growing since 2006, and currently exports its products to several European countries.

As of this year, Bedrocan has set up a separate R&D group to accelerate and stimulate further research on dosing, administration forms, and clinical proof of safety and efficacy. The goal of Bedrocan R&D is to develop tools and materials needed for (clinical) researchers to show the fullest potential of cannabis and its constituents. Projects include, among others, the development of a method to make cannabis placebo, a standardized dosing and administration form for standardized herbal cannabis, and studies into the interaction of cannabinoids and terpenes on cancer cells.

This presentation will give an overview of the current status of the Bedrocan cultivation and product development program. Subsequently, the products and tools under development by Bedrocan R&D will be presented. Participants of the IACM conference 2011 are encouraged to discuss their needs and ideas for research with Bedrocan staff.

FIRST IN HUMAN TRIAL OF AN ORAL TABLET WITH Δ9-THC (NAMISOL®)

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Background: Δ9 -tetrahydrocannabinol (Δ9-THC), the major constituent of the *cannabis sativa* plant, may have medicinal value for certain diseases (Hazekamp and Grotenhermen, Cannabinoids 2010;5,1-21). The efficacy and effectiveness of Δ9-THC in spasticity and pain in multiple sclerosis (MS) has been clearly evidenced in randomized controlled trials. This evidence has been summarized in a meta-analysis resulting in positive conclusions on its efficacy and effectiveness (Iskedjian *et al.*, Curr Med Res Opin 2007;23(1),17-24). In addition, Δ9 -THC may have beneficial effects on neuropathic and chronic pain and on neuropsychiatric symptoms in patients with dementia. Namisol® is a novel formulation of Δ9-THC using Echo Pharmaceutical's propriety drug delivery technology (Alitra™). Alitra™ is an emulsifying technology developed to improve the bioavailability of lipophilic compounds in humans.

Objective: To investigate the route of dosing, safety, tolerability, pharmacokinetics and pharmacodynamics of Namisol®.

Methods: A randomized placebo controlled phase I study was performed in healthy male and female volunteers. The study consisted of two panels. In panel 1 the route of administration was investigated. Using a two-way double-dummy design an oral administration of Namisol® was compared to a sublingual administration of Namisol® (n=12). In panel 2 dose levels of Namisol® were escalated to 8 mg of in a three-way placebo controlled design (n=9).

Results: After oral administration of Namisol® peak levels of Δ9-THC were observed after 30 to 45 minutes. The AUC_{∞} of Δ9 -THC was dose proportional and T_{max} and $T_{1/2}$ were similar for all doses after oral administration of Namisol®. After a dose of 8 mg of Δ9-THC an average increase in VAS feeling high of 0.256 log mm was observed (95% CI 0.093, 0.418). The average increase in heart rate after a dose of 8 mg of Δ9-THC was 5.6 bpm (95% CI 2.7, 8.5 bpm). No treatment related serious adverse events occurred.

Conclusion: Namisol® is safe, is well tolerated and has promising PK/PD characteristics. Based on PK and PD results and practical reasons, the oral route was considered most favorable. Compared to other studies using different formulations of oral THC and nabilone, Namisol® shows smaller variability and a shorter T_{max} (Davis, Expert Opin Investig Drugs 2008;17(1),85-95; Schwilke *et al.*, Clinical Chemistry 2009;55(12),2180-2189). Therefore, Namisol® dose regulation is expected to be easier and Namisol® is expected to give quicker clinical effects compared to registered oral cannabinoids. Clinical Phase II studies are currently ongoing to investigate the effect of Namisol® on spasticity and pain in MS patients, on behavior disturbances in patients with dementia, and on pain in chronic pancreatitis patients.

PRACTICAL ASPECTS OF A TREATMENT WITH DRONABINOL (THC)

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Dronabinol is the primary psychoactive compound in botanical cannabis (Cannabis sativa L.). It is the international non-proprietary name (INN) for (-)-trans-delta-9-tetrahydro-cannabinol (Δ^9-THC or THC). Dronabinol may be extracted from the plant, gained by isomerisation of cannabidiol (CBD) or fully synthesized (as in Marinol).

Administration: Dronabinol can be administered by mouth (oral, sublingual or buccal) or pulmonary (inhaled with a vaporizer, aerosolized or nebulised). Other routes of administration have been used in studies (rectal, intravenously, topically (ophthalmic application), and transdermal).

Bioavailability: Systemic bioavailability of dronabinol after inhalation from cannabis in a pipe was reported to be about 45%, so that it can be expected that inhalation of dronabinol in an alcoholic solution with a vaporizer may result in a similar bioavailability. Oral administration resulted in a mean systemic bioavailability of 6 (range: 4-12%) or 7% (range: 2-14%) in two studies. However, with oral use high amounts of the metabolite 11-hydroxy-THC are formed, which has a similar effect as THC. The pharmacokinetics following buccal administration is similar to that with oral administration.

Dose finding: Usually, oral treatment with dronabinol should be started with once or twice 2.5 mg daily and then slowly increased every second day 2.5 mg until desired therapeutic effects set in or side-effects are observed. Mild side effects may disappear within a few weeks without dose adjustment. A daily dose of 5 mg may be sufficient to increase appetite. Other indications may need 10 to 20 mg. In a clinical study with patients suffering from spasticity due to a spinal cord injury effective daily doses ranged between 15 and 60 mg. In children, dosing can be started with 0.1 mg/kg body weight. According to case reports from a German university mean dronabinol dose was 0.2 mg/kg bodyweight in children with spasticity and pain after finishing dose finding.

Side effects: The most frequent adverse effects of cannabis and THC in clinical studies comprise effects on psyche and cognition (euphoria, dizziness, anxiety, sedation, depression etc.) and dry mouth. There was little difference in side effect profiles between an oral cannabis extract (Cannador) and THC (Marinol) and smoked cannabis vs. dronabinol. Compared to a short-term study in patients with multiple sclerosis a long-term therapy with cannabis and THC over a course of 12 months resulted in a dramatic reduction of adverse effects. In a long-term study the incidence of side effects was no longer higher in the verum groups (dronabinol and cannabis) compared to the placebo group except for the events "dizzy or light-headedness" and "falls."

Tolerance: Clinical long-term studies with dronabinol in patients suffering from multiple sclerosis and AIDS did not find tolerance to the medicinal effects of moderate doses of dronabinol (usually 5-25 mg daily) within 6-12 months.

Withdrawal: As with tolerance, withdrawal symptoms are dose-dependent. In experimental studies comparatively high doses were administered to volunteers (80-120 mg and 210 mg daily, respectively). After discontinuation of dronabinol administration a considerable number of participants experienced irritability, restlessness, insomnia, anorexia, nausea, sweating, salivation, increased body temperature, altered sleep, tremor, and weight loss. In a study with subjects, who used both cannabis and tobacco, withdrawal severity associated with cannabis alone and tobacco alone was of a similar magnitude. Withdrawal symptoms are often absent after discontinuation of therapeutic dronabinol doses.

CIRCULATING ENDOCANNABINOIDS ARE RELATED TO BLOOD PRESSURE IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

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Obstructive sleep apnea chronically increases blood pressure through sympathetic nervous system activation. In animals, arterial hypertension and sympathetic activity are restrained by cannabinoid type 1 receptor activation. Therefore, we hypothesized that increased blood pressure in patients with obstructive sleep apnea is associated with increased circulating endocannabinoid concentrations.

Methods: Arterial oxygen saturation and apnea/hypopnea episodes were recorded in 29 patients with normal glucose tolerance and 26 well matched patients with type 2 diabetes mellitus in the sleep study lab. On the first day, seated blood pressure, insulin, glucose, and high-sensitive CRP were determined in the morning. Insulin sensitivity was measured by euglycemic-hyperinsulinemic clamp on the next day. Markers of systemic inflammation (e.g. IL-6 and hs-CRP) were determined by ELISA. Anandamide, the sum of 1- and 2-arachidonoylglycerol, and oleoylethanolamide were measured in plasma by LC-MS/MS.

Results: **1)** Endocannabinoid concentrations in sleep apnea patients were increased compared to a healthy control group. **2)** Correction for variables of obesity and insulin resistance completely abrogated the difference in endocannabinoids between sleep apnea patients and control subjects. **3)** Anandamide strongly correlated with blood pressure in sleep apnea patients ($r = 0.59$ for systolic and $r = 0.58$ for diastolic blood pressure, $p < 0.001$). **4)** In a multivariate regression analysis, anandamide was a much stronger determinant of blood pressure than sleep apnea severity, obesity, insulin resistance, and inflammation among others.

Conclusions: Blood pressure in obstructive sleep apnea patients correlated with peripheral anandamide concentrations. The relationship cannot be explained by the degree of adiposity, severity of sleep apnea, insulin resistance, or inflammation, which in previous studies were identified as confounding factors for increased endocannabinoid tone and sleep apnea pathophysiology. Based on our findings on peripheral anandamide concentrations in sleep apnea patients, we speculate that increased anandamide may attenuate a further increase in blood pressure, thus, serving as a paracrine or autocrine hemodynamic buffer. Our data suggest a previously not recognized role of the endocannabinoid system for blood pressure regulation in patients with high risk for hypertension and cardiovascular disease. Whether the putative blood pressure effect of anandamide in sleep apnea patients only results from peripheral actions, or presents a combination of central and peripheral hypotensive mechanisms, remains to be elucidated.

WEED AGAINST ZIT? CANNABIDIOL INHIBITS LIPID SYNTHESIS OF HUMAN SEBOCYTES BOTH *IN VITRO* AND *IN SITU*

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Introduction: We have previously shown that locally produced endocannabinoids (e.g. anandamide [AEA]), via auto- and paracrine regulation, play an important role in the maintenance of basal sebum synthesis in human sebocytes through a CB2 receptor-coupled signaling pathway (Dobrosi et al, *FASEB J*, 2008, **22:**3685-3695). Although we have confirmed that human sebocytes have a functionally active endocannabinoid system, we lack data about the potential effect(s) of plant-derived cannabinoids. Therefore, in the current study we have investigated the effects of cannabidiol (CBD) on the viability and lipid synthesis of the sebaceous gland-derived human, immortalized SZ95 sebocytes.

Methods and results: First we have demonstrated that non-cytotoxic concentrations (≤10 μM; determined by MTT-assay and combined $DilC_1(5)$-SYTOX Green staining) of CBD did not affect basal lipid synthesis (Oil Red O and Nile Red staining) of the SZ95 cells, however they dose-dependently prevented the lipogenic effect of various substances (AEA, arachidonic acid, linoleic acid-testosteron combination). Using HPLC-TOF MS we have shown that this universal sebostatic effect is not only a quantitative decrease in the sebum synthesis but a qualitative normalization of the sebocytes' lipidome. Finally, using *in situ* human skin organ culture, we have confirmed that the sebostatic effect develops under "*in vivo-like*" circumstances as well (Oil Red O staining).

As the sebostatic action was markedly suppressed by the decrease of $[Ca^{2+}]_{EC}$, we have investigated how CBD affected the Ca^{2+} homeostasis of the sebocytes. Using a fluorimetric Ca^{2+}-imaging technique (Fluo-4 AM - FLIPR) we have demonstrated that CBD increased the $[Ca^{2+}]_{IC}$ of the sebocytes. It is well described that CBD is able to activate some, mostly Ca^{2+}-permeable, transient receptor potential (TRP) channels. Among the possible TRP-s, we showed the expression of TRPV1, TRPV2 and TRPV4 both at mRNA (Q-PCR) and protein (Western blot, immunocytochemistry) levels. The results of pharmacological blockade and selective "gene silencing" of these channels suggest that the target molecule of the CBD is most probably TRPV4. Moreover, administration of a TRPV4-selective agonist (GSK1016790A) was able to completely mimic the sebostatic action of CBD.

Conclusions: Collectively, our findings suggest that CBD is a novel, very effective sebostatic agent. Our results showing its potent sebum synthesis lowering effect in an *in situ* model system argue that CBD could be successfully applied in the treatment of such a common skin disorder as acne vulgaris, which is characterized by the pathologically elevated sebum production of the sebaceous glands.

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DEMONSTRATING PERIPHERAL RESTRICTION OF CB1 ANTAGONIST TM38837 IN HUMANS

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Introduction: Cannabinoid 1 receptor (CB_1) antagonists show beneficial effects in metabolic disorders. However, psychiatric side effects of first generation brain penetrating compounds have led to market withdrawal of rimonabant and cancellation of several development programs. The side effects are mediated by central CB_1, while there are indications that the beneficial effects are peripherally mediated. TM38837 is a novel CB_1 antagonist that pre-clinically showed negligible penetration into the central nervous system, whereas weight loss and metabolic effects comparable to rimonabant were observed. In the current study, TM38837 was studied in healthy male subjects, using a 9-delta tetrahydrocannabinol (THC) challenge test with rimonabant as a positive control. Different validated tests were used to probe the reversal of peripheral and central effects of THC.

Methods: This was a double-blind, double dummy, randomized, placebo-controlled, cross-over, partial parallel study. During occasions 1 to 4, 24 subjects were treated with TM38837 100 mg, 500 mg, placebo TM38837 or placebos only. During occasion 5, subjects received either rimonabant 60 mg or placebo rimonabant, both combined with placebo TM38837. Five THC 4 mg inhalation challenge tests over two days were performed during each occasion. Blood samples were drawn for pharmacokinetic (PK) analyses of TM38837, rimonabant and THC. Body sway, visual analogue scales (VAS) by Bond & Lader (mood, alertness and calmness) and Bowdle (psychedelic effects), and heart rate were assessed frequently as pharmacodynamic (PD) measures.

Results: THC particularly affected feeling high, body sway, and heart rate. These effects were partly antagonized by Rimonabant 60 mg and TM38837 500 mg; whereas the 100 mg dose of TM38837 had no measurable impact on VAS feeling high and body sway and only limited effect on heart rate.

Conclusions: Rimonabant 60 mg showed a larger antagonizing potential on all THC-induced effects than TM38837 500mg, except for heart rate where the antagonizing effect was considered to be similar. The 100 mg dose of TM38837 had no measurable impact on THC-induced CNS-effects, suggesting that this dose does not penetrate the brain. TM38837 has been shown to be equipotent to rimonabant with regard to weight loss and other metabolic effects in rodent obesity models; hence these results are very encouraging for further development of TM38837 as a peripherally restricted CB_1 receptor antagonist.

XANTHINE DERIVATIVES AS NOVEL LIGANDS FOR CANNABINOID RECEPORS AND THE RELATED ORPHAN RECEPTOR GPR55

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The diverse physiological effects of D^9-tetrahydrocannabinol (THC), the main bioactive constituent of the C*annabis sativa* plant, are mediated through the activation of cannabinoid (CB_1 and CB_2) G protein-coupled receptors (GPCR). While the CB_1 receptor is predominantly expressed in the CNS, the CB_2 receptor can primarily be found on immune cells. Recently, cannabinoids as well as the CB_1 receptor inverse agonist rimonabant were found to activate the orphan GPR55 at micromolar concentrations, raising the question whether the GPR55 receptor might be a third cannabinoid receptor subtype. However, only very little is known so far about GPR55 and its ligands. Therefore potent ligands are required to elucidate the physiological role of GPR55 and its potential as a novel drug target. We have designed, synthesized and pharmacologically characterized a series of xanthine derivatives as CB and GPR55 receptor ligands, and were successful in obtaining CB_2 receptor selective agonists and GPR55 antagonists.

Methods: Compounds were tested for affinity in heterologous radioligand binding experiments at both human CB receptor subtypes. Compounds showing a K_i value of <1 µM were funcionally characterized in cAMP accumulation assays. Interaction with the GPR55 receptor was investigated by measuring β-arrestin translocation to the activated receptor, which was detected by measuring luminescence emission, based on β-galactosidase enzyme fragment complementation technology (β-arrestin PathHunter™ assay, DiscoverX).

Results: **1)** Some of the synthesized compounds show high CB_2 receptor affinity and selectivity. **2)** Bulky aliphatic residues in position 1 of the xanthine scaffold are preferred over aromatic or short aliphatic moieties. **3)** The most potent compound so far possesses affinity in the nanomolar range (K_i: 357 nM). **4)** Further pharmacological investigation revealed agonistic properties in cAMP accumulation assays. **5)** First results indicate that N1-benzyl substituted compounds possess antagonistic properties at GPR55.

Conclusions: **a)** The xanthine scaffold – a privileged structure in medicinal chemistry - is suitable for the development of CB and GPR55 receptor ligands. **b)** Novel CB_2-selective ligands with agonistic properties could be obtained. **c)** Antagonists at GPR55 could be identified which show selectivity versus CB receptors. **d)** The identified compounds provide a basis for the development of more potent and selective ligands at CB receptors and the GPR55.

BEYOND CANNABIS AND ANANDAMIDE.

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Nearly 50 years ago, Gaoni and I isolated the psychoactive constituent of Cannabis, Δ^9 – tetrahydrocannabinol (THC), elucidated its structure and synthesized it. Although a huge amount of research was done on THC its mechanism remained obscure for almost 20 years, when Allyn Howlett's group in the US, found a specific brain receptor, named CB1, whose stimulation led to the well known marijuana effects. Then, in the early and mid 1990's, we identified the endogenous cannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG) that act on CB1. The biosyntheses and metabolism of these endocannabinoids were clarified by efforts of many groups in the US and Europe. Thus, the endocannabinoid system became a reality and was found to be involved in a large number of physiological processes.

I shall discuss 2 major aspects of endocannabinoid activity:

1. **Specific CB2 receptor agonists**. These agonists do not cause marijuana-type activity, but act as major novel protective entities in a variety of pathological conditions. The activity of a novel, camphor-resorcinoid HU-910 in lowering the formation of pro-inflammatory cytokines and in protection of brain trauma will be discussed.

2. **Endogenous N-acyl amino acids and related constituents**. Anandamide is the amide of arachidonic acid with ethanolamine. It can be viewed as the forerunner of a many additional fatty acid – amino acid (or amino acid-derived) molecules in the brain, as well as in the periphery. The activity of most of these novel entities remains to be elucidated. Exploratory work on this family of endogenous constituents has however revealed that some of these N-acyl-amino acids play a role in diverse biochemical systems. A few examples:

Arachidonoyl serine – causes vasodilation and is .neuroprotective after traumatic brain injury by reducing apoptosis.

Oleoyl serine **(OS)** - In a mouse ovariectomy model for osteoporosis, Itai Bab has shown that OS effectively rescues bone loss by increasing bone formation and markedly restraining bone resorption.

A few examples from other groups: palmitoyl ethanolamide is anti-inflammatory and anti-epileptic; arachidonoyl glycine lowers pain; oleoylethanolamide suppresses feeding.

Conclusion. Synthetic specific CB2 agonists and endogenous N-acyl-amino acids (and related moieties) seem to be major groups of cannabinoid-like compounds with a wide spectrum of physiological activities.

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POSTER ABSTRACTS

(1) AGE-RELATED CHANGES IN THE ENDOCANNABINOID LEVELS, OXIDATIVE STRESS AND LYSOSOMAL ACTIVITY IN MICE LACKING CB1 RECEPTOR

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Genetic deletion of cannabinoid 1 (CB1, Cnr1) receptor in mice has been shown to accelerate age-related memory decline and neuronal loss, the cause of which has not been identified yet. Increased oxidative stress and accumulation of metabolic end products, like aging pigment lipofuscin, as well as decrease in lysosomal function and autophagy deficits have been shown to contribute to aging. Thus, we sought to test if CB1 knockout mice suffer from increased oxidative stress and have decreased lysosomal and autophagic activity. Additionally, we investigated changes in the endocannabinoid levels in aging, in particular 2-arachidonoylglycerol (2-AG), since it is known to have neuroprotective effects that are only partially dependent on the CB1 receptor.

Methods: 2-, 5- and 12-month-old CB1 receptor knockout mice and their WT littermates on a C57BL/6J background were used for this study. We first investigated the presence of oxidized macromolecules (lipids, proteins, DNA) in the hippocampus using colorimetric assays for lipid and protein oxidation and immunostaining for 8-hydroxyguanosine as a read-out for oxidative DNA damage. Lipofuscin accumulation was measured as the intensity of typical yellow-brownish autofluorescence in the tissue that increased with age. Cathepsin D and LC3 expression was assessed by Western Blot analysis. 2-AG levels were measured with LC/MS.

Results: We found a similar age-related increase in the amount of oxidized lipids, proteins and DNA in both $Cnr1^{-/-}$ and WT mice. Cnr1-/- mice also showed an exacerbated lipofuscin accumulation in the CA3 hippocampal region with increasing age. We found no significant difference in the amount of LC3, marker of autophagosome formation, between the two strains. However, the expression of active cathepsin D, one of major lysosomal proteases, which has been previously implicated in the degradation of oxidized proteins, was significantly decreased in the hippocampus of CB1 knockout mice. 2-AG levels in the hippocampus of WT mice were shown to decrease with age but increase in the Cnr1-/- mice.

Conclusions: Significant reduction in cathepsin D expression in the Cnr1-/- mice could explain increased lipofuscin accumulation in the hippocampal neurons and contribute to their degeneration. The mechanism by which CB1 receptor signalling influences cathepsin D expression and activation remains to be elucidated. An increase in 2-AG levels probably represents a compensatory mechanism, since 2-AG can act as a natural antioxidant, and its age-related increase in the CB1 knockout mice could be the reason why there was no significant difference found in the amount of oxidized macromolecules between the two strains.

(2) ROLE OF CANNABINOID 1 RECEPTORS ON HIPPOCAMPAL GABAERGIC NEURONS IN BRAIN AGING

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Brain aging is associated with cognitive decline accompanied by progressive neuroinflammatory changes. It is suggested that the age-dependent increase in neuroinflammation substantially contributes to age-related memory deficits. However, the molecular and cellular mechanisms that might contribute to these processes are still unknown. The endocannabinoid system is involved in the regulation of glial activity therefore we asked whether the early cognitive decline in animals lacking cannabinoid 1 receptors ($Cnr1^{-/-}$) is associated with enhanced neuroinflammation.

Methods: Experiments to test the consequence of a constitutive deletion of the CB1 receptor were carried out with male and female $Cnr1^{-/-}$ and $Cnr1^{+/+}$ littermates on a congenic C57BL6/J background. The conditional GABA and glutamate-specific knockout animals were kept on a predominant C57BL/6N genetic background. The spatial learning and memory abilities of mice were assessed in the Morris water maze. GFAP-immunoreactive astrocytes were analyzed using area fraction technique, whereas the optical fractionator method was used to quantify the total number of Iba1-immunoreactive microglia, and NeuN-positive neurons. Total RNA was extracted from the isolated hippocampi and mRNA expression was determined.

Results: The age-dependent increase in astrocyte and microglia cell numbers, the ratio of CD40 expressing activated microglia and the interleukin-6 levels were elevated in the $Cnr1^{-/-}$ mice. This enhanced glial activity was accompanied by a loss of principal neurons in the hippocampus and deficits in spatial learning. Deletion of CB1 receptors from the forebrain GABAergic, but not from the glutamatergic neurons, led to similar age-related changes as observed in the constitutive knockout animals.

Conclusions: In the present study, we show that reduced CB1 receptor signaling impairs the glial control by GABAergic cells. The resulting enhanced neuroinflammation, elevation of IL-6 levels further aggravate GABAergic neuronal activity in the hippocampus leading to further deficits in glial control, progression of pathological changes and thus to an early onset of age-related changes in the brain. Our results suggest that CB1 receptor activity on hippocampal GABAergic neurons is necessary for glial activity regulation, for protection against neuroinflammation and thus against age-dependent cognitive deficits.

(3) MULTITARGET DRUGS AT CB2 RECEPTOR AND BuChE FOR ALZHEIMER DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder in the central nervous system (CNS) causing a progressive deterioration in cognitive functions. The disease is characterized by neuropathological lesions, manifest as protein deposits known as b-amiloid plaques and interneuronal tangles formed by neurofibres consisting of coiled filaments of tau protein. Among the main therapeutic strategies used in AD stands out that based on the inhibition of brain cholinesterase activity. Recent evidences suggest that butirylcholinesterase (BuChE) can also hydrolises acetylcholine in the brain and may play a role in cholinergic transmission (Martínez Gil, A. *Medicinal Chemistry of Alzheimer´s disease*. **2009**; Campillo N.E.; Páez J.A. *Mini Rev. Med. Chem.* **2009**, 9 (5*),* 539-59).

Alterations in components of the cannabinoide system have been reported in Alzheimer´s patients, suggesting that the cannabinoide system either takes part or is altered by the pathophysiology of the disease. In AD patients the cannabinoid receptors have found to be abundantly expressed in neuritic plaque-associated astrocytes, and selectively expressed in microglia. Many evidences reveal that cannabinoids mediate suppression of inflammation *in vitro* and *in vivo* through stimulation of CB2R (Campillo N.E.; Páez J.A. *Mini Rev. Med. Chem.* **2009**, 9 (5)*,* 539-59; Campbell, V. A.; Gowran, A. *Br. J. Pharmacol* **2007**, 152, 655-662)

The targeting of multiple receptors through a single drug molecule is gaining increasing acceptance. This multifunctional drug paradigm strongly challenges the widely held assumption that single-target agents are better to "dirty drugs" in therapy. Although the single-target approach currently remains the major drug discovery strategy in pharmaceutical companies, there is increasing recognition of the limitations of such an approach for complex diseases (Van der Schyf, C. J.; Youdim, M. B. *Neurotherapeutics* **2009**, *6*, 1-3).

Given the promiscuous behaviour of cannabinoide system in terms of potential targets we envisaged the idea to design multi-target cannabinoids ligands that modulate two targets-CB2R and BuChE simultaneously. Thus, the main goal of this work has been to design, by docking techniques, a multi-target set of cannabinoids agonists and inhibitors of BuChE, their synthesis and study their therapeutic application as potential drugs for neurodegenerative diseases. Thus, we have obtained a new family of indazolyl ether derivatives whose behavior as cannabinoid agonists and inhibitors of BuChE make them an innovative strategy for treatment of Alzheimer´s disease (Paéz J.A, Campillo, N.E, González P., Pérez C., Arán V.J., Martín M. I., Girón R., Sánchez E. M. PCT/ES2010/000400. **2010**. Universidad Rey Juan Carlos).

(4) THE COMBINATION OF D^9-THC AND CANNABIDIOL REDUCES THE COGNITIVE IMPAIRMENT IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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The reduced effectiveness of current therapies against Alzheimer's disease (AD) triggers the need for intensifying the research efforts devoted to develop new agents for preventing or retarding the disease process. Targeting the endocannabinoid system could offer a multi-faceted approach for the treatment of AD since previous evidence indicate that cannabinoids provide neuroprotection by reducing neuronal loss, neuroinflammation and oxidative stress as well as by promoting the brain's intrinsic repair mechanisms. Sativex® is the first cannabinoid-based medicine recently approved in the UK, Canada and Spain for the treatment of spasticity due to multiple sclerosis. The two main components of Sativex® are delta-9-tetrahydrocannabinol (D^9-THC) and cannabidiol (CBD). The aim of the present study is to test the potential therapeutic properties of D^9-THC and CBD in APP/PS1 mice. Double transgenic mice expressing mutant amyloid precursor protein (APPswe) and mutant presenilin 1 (PS1-dE9) are a valuable AD model because develop cognitive impairment, progressive brain b-amyloid deposits and other AD-related neuropathological alterations starting by the age of the 6 months.

Methods: We administered a non-psychoactive dose of D^9-THC (0.46 mg/kg, i.p.) and CBD (0.43 mg/kg, i.p.) or vehicle once daily during 5 weeks to APP/PS1 mice and wild-type littermates aging 6 months at the beginning of the study. After a period of washing, memory performance on the two-object recognition test and learning capacity on the active avoidance test were evaluated in mice. The burden of b-amyloid deposits and glial responses were analysed in brains samples by immunohistochemical techniques.

Results: **1)** The combination of D^9-THC and CBD reduced the memory and learning impairment exhibited by APP/PS1 mice at 8 months of age. **2)** Immunohistochemical studies revealed that the burden of b-amyloid deposits in the cortex of chronically treated APP/PS1 mice was not significantly modified, in spite of a tendency to reduction in D^9-THC and CBD-treated animals. **3)** The treatment with D^9-THC and CBD reduced the astrogliosis in the b-amyloid surrounding area in APP/PS1 mice. However, **4)** the microglial response associated to b-amyloid deposition was preserved in D^9-THC and CBD-treated animals.

Conclusions: These results suggest that **a)** the combination of D^9-THC and CBD reduced the cognitive impairment in an animal model of AD. **b)** The mechanisms related to astroglial response could underlie the described effects of cannabinoids on this model. Thus, **c)** Sativex® could be considered as a potential therapeutic strategy against AD.

(5) CANNABINOID RECEPTOR 1 (CB1) KNOCK-OUT AFFECTS BIOCHEMICAL PROCESSING OF THE AMYLOID PRECURSOR PROTEIN (APP) AND LEADS TO A DECREASE IN COGNITIVE ABILITIES IN A MOUSE MODEL OF ALZHEIMER'S DISEASE (AD)

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Dementia is strongly associated with ageing and therefore the numbers of dementia patients will significantly increase in industrial countries as their populations are ageing. The most common form of dementia is Alzheimer's disease (AD). AD is characterized by extracellular amyloid ß (Aß)-deposition, intracellular neurofibrillary tangles and glial activation, accompanied by selective neuronal loss and cognitive deficits. Endocannabinoids can display neuroprotective effects and affect neuronal plasticity and memory. Brain samples from AD patients showed a reduced number of CB1-expressing neurons in brain areas affected by amyloid plaque deposition and microglial activation. Injection of cannabinoids in Aß-treated rats prevented glial activation, neuronal loss and cognitive deficits. These findings suggest an involvement of the endocannabinoid system (ECS) in pathogenesis of AD and prompted us to study the involvement of the ECS in the development of AD by establishing a new mouse model by breeding APP23 mice, a well-described AD transgenic mouse model, with CB1-deficient mice. APP23/CB1-/- mice showed a lower birthrate and reduced bodyweight as well as a higher mortality rate compared to APP23/CB1+/+ and CB1-/- mice. In addition APP23/CB1-/- mice showed impaired learning and memory compared to APP23/CB+/+ and CB1-/- mice, analysed by Morris-water-maze (MWM). Surprisingly, these detrimental effects were not accompanied by an increased Aß-plaque load but rather by a reduction of amyloid plaques. Biochemical analysis exhibited reduced APP-processing and gliosis in CB1-deficient mice. To reveal the molecular interplay between CB1 and APP, we generated transgenic neuronal cell lines (N2A cells) expressing endogenous APP and overexpressing CB1. Overexpressing CB1 led to elevated levels of APP-processing products, whereas CB1 siRNA knock-down showed a reduction in APP cleavage product formation. Furthermore we found, that this effect of CB1-signalling on APP-processing is dependent on activation of the MAP-kinase pathway. Thus, the direct interplay of CB1 and APP may act as a novel pharmacological target for prevention and treatment of AD patients.

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(6) ANXIOLYTIC-LIKE PROPERTIES OF ST4070, A NOVEL, POTENT AND SELECTIVE REVERSIBLE INHIBITOR OF FAAH

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Introduction: Current management of anxiety, a common and debilitating disorder with a high social and personal cost, is far from satisfactory. Therefore, in the last years an urgent need for novel pharmacological approaches has emerged. One such strategy involves targeting the endocannabinoid system (ECS), and there is now considerable evidence for the central role played by ECS in coping with the regulation of stress and emotions. More recently, attention has moved from directly targeting type-1 cannabinoid (CB_1) receptors to indirectly enhancing the content of their endogenous ligands, a more valuable strategy that preserves the spatiotemporal specificity of endocannabinoid activity. Despite recent evidence suggesting the benefits of inhibiting fatty acid amide hydrolase (FAAH), the enzyme that degradates the endogenous CB_1 agonist arachidonoylethanolamide (anandamide), further research is needed to demonstrate the therapeutic efficacy of FAAH inhibitors, and their putative translation into the clinic.

Methods: ST4070, a novel, potent and selective reversible inhibitor of FAAH, was administered *per os* in CD1 male mice (3 to 30 mg/10 ml/kg), that were tested in the elevated-plus maze, and in Wistar male rats (3 to 30 mg/2.5 ml/kg), that were tested in the light-dark apparatus. In addition, the effect of ST4070 on FAAH activity and on the content of FAAH substrates (anandamide and palmitoylethanolamide) in selected brain regions was assessed by radiochromatography and LC-MS analysis.

Results: ST4070 showed clear anxiolytic-like properties in both rodent models, and in parallel it also produced a clear inhibition of FAAH activity, and a significant increase of anandamide and palmitoylethanolamide in behaviorally-relevant brain regions.

Conclusions: Investigation into novel pharmacological targets for the management of anxiety holds the promise to further our understanding of its aetiology, and to develop new and more effective anxiolytic drugs, like the reversible FAAH inhibitor ST4070.

(7) A STRUCTURE-AFFINITY STUDY ON NEW RESORCINOL "HYBRIDS": ROLE OF THE AMIDE HEAD ON THE CANNABINOID RECEPTOR INTERACTION

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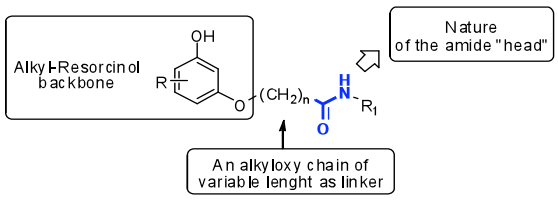
The discovery of cannabinoid receptors (CBs) and their putative ligands (i.e. endocannabinoids) less than twenty years ago has open the way for the identification of a plastic innate signalling system, better known as the Endocannabinoid System (ES), involved in many (patho)physiological functions. Thus, there has been a growing interest in the development of new compounds that selectively regulate and/or modify the action and the levels of these lipid mediators.
During our research in the cannabinoid field, we have designed and developed an attractive medicinal chemistry template (*Brizzi et al., J.Med. Chem. 2005; 48:7343-7350*) which turned out to produce potent cannabimimetic ligands. In our previous studies, we have also assessed the influence of both the alkyloxy chain (*Brizzi et al., Bioorg. Med. Chem. 2007, 15:5406-5416*) and the alkyl tail on the cannabinoid receptor affinity (*Brizzi et al., J. Med. Chem.*



2009, 52:2506-2514). In order to validate our resorcinol-hybrid model, we have synthesized a novel series of compounds characterized by an amide head with different electronic properties or a glycerol-ester/glycerol-ether head.
Methods: The synthetic pathway started from properly substituted phenols (i.e.olivetol, 5-(2-methyloctan-2-yl)resorcinol, 4-hexylresorcinol) which were alkylated with bromoalkyl acid methyl esters and/or bromoalkyl glycerol-esters/ethers in dry acetone in the presence of anhydrous potassium carbonate and potassium fluoride to furnish the corresponding esters/ethers in moderate to good yield. Final amides were synthesized reacting the intermediate acid, obtained from hydrolysis of esters with methanolic/aqueous sodium hydroxide solution, with the appropriate amines in the presence of different condensing agents. Glycerol-derivatives were obtained by cleavage of the benzylidene and acetonide protecting groups. All the newly synthesized compounds were evaluated for their affinity at the recombinant human CB_1 and CB_2 receptors over-expressed in COS cells.
Results: 1) The amide head should be have a small steric hindrance; 2) Substitution of the amide by an ester head was well tolerated, giving potent CB_1 ligands; 3) Inversion of the amide head caused a decrease or a loss of the receptor activity; 4) Replacement of the carbonyl moiety with a methylene group (ether linkage) proved to be detrimental for cannabinoid receptor interaction.
Conclusions: Analysis of the binding assay results was very complex and multifaceted. The carbonyl group seems to play a crucial role in the cannabinoid receptor interaction and these resorcinol hybrids appears to be very sensitive to structural changes in the amide moiety.

(8) EFFECTS OF CANNABIDIOL AND OTHER PHYTOCANNABINOIDS ON SEROTONIN 5-HT$_{1A}$ RECEPTORS

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Cannabidiol (CBD) is, together with Δ^9-Tetrahydrocannabinol (THC), one of the most studied constituents of the plant *Cannabis Sativa*. Unlike THC, CBD does not cause psychotropic effects. Recently, Parker's group reported that CBD (5 mg/kg) is able to suppress conditioned gaping in rats and vomiting in shrews, and that this suppression is opposed by the phytocannabinoid cannabigerol (CBG) (Rock et al., 2011) which we had shown previously to behave as a 5-HT$_{1A}$ receptor antagonist *in vitro* (Cascio *et al.*, 2010). Together, these findings support the hypothesis that the anti-nausea/anti-emetic effects of CBD are mediated by somatodendritic 5-HT$_{1A}$ receptors in the dorsal raphe nucleus (Parker et al., 2011). In 2005, Russo *et al*. obtained *in vitro* evidence that CBD behaves as a direct agonist at serotonin 5-HT$_{1A}$ receptors, albeit at rather high concentrations (EC_{50}= 16 μM). In the present study, using rat brainstem membranes, we evaluated the ability of CBD to interact with 5-HT$_{1A}$ receptors at lower concentrations.

Methods: Displacement binding assays with tritiated 8-OH-DPAT were performed using rat brainstem membranes. [^{35}S]GTPgS binding assays were performed using either rat brainstem or MF1 mouse whole brain membranes.

Results: We found that: **1)** in displacement binding assays, CBD, at concentrations ranging from 1 nM to 10 μM, did not induce any detectable displacement of tritiated 8-OH-DPAT and **2)** in [^{35}S]GTPγS assays, CBD up to 10 μ M did not show any signs of 5-HT$_{1A}$ receptor agonism. Moreover, **3)** neither 10 nM nor 1 μ M CBD modulated 8-OH-DPAT-induced activation of 5-HT$_{1A}$ receptors in brainstem membranes. In contrast, however, at 100 nM, CBD markedly enhanced this activation, raising the possibility that 100 nM CBD may target an allosteric site on 5-HT$_{1A}$ receptors. This possibility is currently under investigation. We also evaluated the effect of CBD on 8-OH-DPAT agonism, using MF1 mouse whole brain membranes. Interestingly, **4)** we found that CBD, at 100 nM, causes a significant reduction of 8-OH-DPAT efficacy. At the same concentration, **5)** CBDV, the propyl analogue of CBD, also reduces 8-OH-DPAT efficacy in mouse whole brain membranes. In contrast, however, **6)** 100 nM Δ^9-tetrahydrocannabivarin (THCV) and 100 nM CBG antagonize 8-OH-DPAT without reducing its efficacy, suggesting that they act as competitive antagonists of this 5-HT$_{1A}$ receptor agonist.

Conclusions: Our results suggest that CBD, CBDV, CBG and THCV can all modulate 5-HT$_{1A}$ receptor activation with significant potency. Further experiments are now underway to investigate the effect of these phytocannabinoids on 8-OH-DPAT agonism in different rat and/or mouse 5-HT$_{1A}$ receptor-containing brain areas, as well as in cells transfected with human 5-HT$_{1A}$ receptors.

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(9) TONIC INHIBITORY EFFECT OF ENDOCANNABINOIDS ON HUMAN CONNECTIVE TISSUE- AND MUCOSAL- TYPE MAST CELL FUNCTIONS IN SITU VIA CANNABINOID RECEPTOR 1 (CB1) SIGNALING

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Introduction: Since many chronic inflammatory and allergic disorders are characterized by excessive mast cell (MC) numbers and activation, it is clinically important to understand the physiological controls that avoid increased MC numbers/degranulation in normal human tissues. Recently, cannabinoids have surfaced as important neuroendocrine regulators of MC biology. Previously, we have shown that locally produced prototypic endocannabinoids (e.g. anandamide, AEA) markedly inhibit human hair follicle (HF) growth via cannabinoid receptor (CB) 1 (Telek et al, FASEB J 2007).
Methods: Now, we have investigated how CB1-signaling affects human MCs *in situ*, focusing on connective tissue-type MCs (CTS-MCs) in the connective tissue sheath (CTS) of organ-cultured scalp HFs and on mucosal-type MCs (M-MCs), using organ-cultured nasal polyps (NP) as a surrogate tissue for human bronchial mucosa.
Results: We show that Kit+ CTS- and NP-MCs express functional CB1 receptors *in situ*. Blockade of CB1-signaling (using the specific CB1 antagonist AM251 or CB1 gene knockdown by siRNA) significantly enhanced CTS- and M-MCs degranulation and increased their total number *in situ*, as shown by quantitative MC (immuno-)histochemistry and ultrastructurally. Strikingly, inhibiting CB1-mediated signaling did not promote Kit+ CTS-MCs proliferation, but their maturation *in situ* from resident progenitor cells in the CTS, probably via up-regulating stem cell factor (SCF) production. Both the endocannabinoid, AEA, and the CB1-selective agonist, ACEA, effectively counteracted the degranulation of MCs by potent endogenous and exogenous MC secretagogues (substance P, compound 48/80) *in situ*.
Conclusions: Thus, in both human skin and airway mucosa, MC activation and maturation from resident progenitors is subject to an important inhibitory endocannabinoid tone. This invites one to target the intracutaneous and intrabronchial endocannabinoid system as a novel strategy in the future management of allergic diseases. Furthermore, we show that the CTS of human HFs and the nasal mucosa of human nasal polyps offer excellent, physiologically and clinically relevant model systems for investigating and manipulating the biology of human MCs within their natural tissue context habitat.

(10) CANNABINOID RECEPTOR TRAFFICKING IN PERIPHERAL IMMUNE CELLS IS TYROSINE PHOSPHATASE-DEPENDENT

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Emerging evidence implicates a role of the endocannabinoid system in a wide variety of physiological and pathophysiological processes, including metabolic regulation, pain, anxiety, immunomodulatory functions and bone growth. Since cannabinoid receptor agonists/antagonists acting at CB1 and CB2 receptors provide tools to interfere in physiological and pathological processes, an understanding of the regulation of CB receptor surface expression is mandatory.

Methods: CB receptor plasma membrane levels of leucocyte subpopulations were monitored by subtype specific fluorescence labeling of cells and flow cytometry. CB2 triggered calcium transients were used to monitor CB2 receptor surface expression induced activation. Refined characterization of receptor membrane localization was carried out in HL60 cells by lipid raft and whole plasma membrane isolation, co-immunoprecipitation and shRNA mediated CB1 receptor knock downs.

Results: In contrast to previous assumptions, CB2 surface expression of primary immune cells and the promyelotic HL60 cancer cell line is not altered by exposure to micromolar concentrations of arachidonoylethanolamide, 2-arachidonoylglycerol and CP55,940. Incubation of cells with different tyrosine-phosphatase inhibitors like phenylarsenoxide, BPA-AM2 and hydrogen peroxide induce either CB2 receptor internalization or externalization in a cell type dependent manner. Cells with high basal surface expression levels (monocytes/macrophages and HL60) display an internalization of the receptor, whereas cells with low basal surface expression recruit receptors from intracellular pools and elevate CB2 surface expression in response to phosphatase inhibitor treatment. In HL60 cells both CB1 and CB2 receptors were detected by SDS page in the lipid raft fraction of the plasma membrane and could be co-immunoprecipitated from whole plasma membrane and from lipid raft fractions, indicating CB1/CB2 heterodimers. In the same cell line shRNA knock downs of Cnr1 decreased CB1 receptor surface expression, which was accompanied by simultaneous reduction of CB2 receptor surface levels to a CB1 matching extent. Furthermore, reduction of CB2 membrane levels after knock down of CB1 in a stoichiometric manner with CB2 suggests that intracellular heterocomplex formation may be a prerequisite for adequate trafficking of CB receptors after synthesis to the plasma membrane in HL60 cells.

Conclusions: In contrast to CB1, which undergoes rapid desenzitation and internali-zation upon agonist treatment in CNS, CB2 surface expression in peripheral immune cells seems to be regulated in an agonist independent manner. We found a “molecular switch” which regulates the trafficking of peripherally expressed CB1 and CB2 from the plasma membrane to intracellular compartments or vice versa, depending on the cell type. This implicates a new potential regulatory mechanism for CB receptor signaling in peripheral immune cells, since receptor surface density is a key regulatory mechanism for G protein-coupled receptor (GPCR) activity, influencing strength and duration of signal transduction into the cell.

(11) FREQUENCY-DEPENDENT MODULATION OF NORADRENERGIC TRANSMISSION BY CANNABINOIDS IN THE RAT PREFRONTAL CORTEX (PFC)

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Previous functional data are controversial how endo- and exocannabinoids regulate monoaminergic transmission in the prefrontal cortex - a brain region implicated in a multitude of neuropsychiatric diseases. The aim of our study was to demonstrate whether modulation of cannabinoid receptors alters basal and electric field stimulation (EFS) evoked [^{3}H]norepinephrine ([^{3}H]NE) release from rat PFC slices. In particular, we were interested in revealing possible interactions with other neuromodulatory mechanisms.

WIN 55212-2, a synthetic cannabinoid receptor agonist, when applied alone was without significant effect on [^{3}H]NE efflux at low frequency stimulation (2 Hz). However, when α2-adrenergic receptors (α2-AR) were inhibited using idazoxan, a selective α2-antagonist - which alone augmented NE release -, the inhibitory action of WIN 55212-2 on stimulation-evoked [^{3}H]NE efflux was exacerbated. Interestingly, this effect was more pronounced when higher stimulation frequency was used (10 Hz), which may indicate changes in fine modulation of the involved signaling pathways. The cannabinoid reuptake inhibitor VDM11 significantly attenuated [^{3}H]NE efflux indicating that endocannabinoids might also activate this regulatory pathway. In the presence of AM251, an inverse agonist of the CB1 receptor, the inhibitory effect of WIN 55212-2 on NE efflux was reversed, indicating that it is mediated by CB1 cannabinoid receptors. Co-administration of idazoxan and WIN to rats leads to significantly enhanced depressive behaviour, as assessed by the forced-swim test which illustrates a probable behavioral implication of the studied mechanism.

α2-AR and CB1 co-localize on noradrenergic varicosities in the PFC which suggests a direct interaction between cannabinergic and noradrenergic transmission here. As both receptors are $G_{i/o}$ coupled, the utilization of common signal transduction machinery or agonist induced heterologous desensitization by receptor internalization could be considered as an explanation of this interaction. Using a N-terminal antibody against the CB1 receptor we demonstrated that the CB1 signal decreased when PFC slices are treated with idazoxan. Furthermore, the inhibitory action of WIN on [^{3}H]NE efflux was partly reversed by application of the wide-spectrum phosphatase inhibitor okadaic acid.

In conclusion, our results show that CB1 receptor activation inhibits norepinephrine release in the prefrontal cortex and this modulation is dependent on stimulation frequency and the autoinhibition of α2 adrenoceptors. Heterologous desensitization of the CB1 receptor by means of a phosphorylation-dependent internalization likely plays a role in this interaction.

(12) INTERACTION OF 2-ARACHIDONOYLGLYCEROL WITH THE $GABA_A$ RECEPTOR COMPLEX

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Introduction:
Within the context of the discussion about the interaction of cannabinoids with the $GABA_A$ receptor complex (1), we determined the influence of 2-Arachidonoylglycerol (2-AG) in in vitro receptor binding studies. [^{3}H]TBOB ([^{3}H]t-butylbicycloorthobenzoate) was used as a tracer ligand.

Methods:
We used well-washed rat brain membranes (2).

Results:
GABA inhibited [^{3}H]TBOB binding according to a one site model with an IC_{50} of 5 mM and a slope parameter not significantly different from unity. 2AG inhibited [^{3}H]TBOB binding according to a one site model with an IC_{50} of 80 mM and a slope parameter not significantly different from unity. The synthetic cannabinoids O2545 and WIN-55,212 also inhibited [^{3}H]TBOB binding in the high micro-molar range. Arachidonic acid, in a concentration up to of $2\ 10^{-4}$ M, did not influence the binding of [^{3}H]TBOB.
The affinity of GABA was also determined in the presence of 50 μM 2AG. Isobolographic analysis, using an endpoint of 50 % [^{3}H]TBOB binding, showed additivity between GABA and 2AG, thus no synergy.

Discussion:
The $GABA_A$ receptor complex comprises the Cl^--channel and binding sites for a variety of compounds. All these sites are allosterically coupled, resulting in a network of interactions, ultimately controlling the ion-channel. The functional state of this channel is reflected in the amount of ligand that binds to the convulsant site. This site can be labelled with [^{3}H]TBOB (2).
Our results show that cannabinoids indeed bind to the $GABA_A$ receptor complex, acting in a GABA agonistic, additive way. Whether the cannabinoids bind to the GABA site itself or to an allosteric site is undetermined. Presumably the binding might explain the physiological effects reported by others groups (1).

1. Baur R, Gertsch J, Sigel E. The cannabinoid receptor CB1 antagonists rimonabant (SR141716) and AM251 directly potentiate GABA(A) receptors. Br J Pharmacol. 2011 Apr 6. [Epub ahead of print]
2. van Rijn CM, Willems-van Bree E. A four-ligand hypercube model to quantify allosteric interactions within the GABAA receptor complex. Eur J Pharmacol. 2004, 485:43-51.

(13) DEFRAGMENTATION OF THE PERIODS OF IMMOBILITY DURING CHRONIC ADMINISTRATION OF PHYTOCANNABINOIDS IN RATS

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Introduction:
Cannabinoids are used throughout the world in various therapeutic applications. Cannabis is commonly believed to induce drowsiness and sleepiness, however, sound scientific evidence is scarce. Moreover, in pre-clinical studies controversial effects have been reported: increases as well as decreases in sleep time are reported (references in 1). In the present study we investigated whether the effects on spontaneous mobility in rats reported for the synthetic cannabinoid agonist WIN55 (R(+)WIN55,212 (2)), also applies for the oil of cannabis strain White Widow.

Methods:
Experiments were performed in male Wistar rats (n=8 per group), age 8-10-months. Movements of freely moving animals were recorded with an analogic passive infrared detector (PIR). Simultaneously, cortical EEG was recorded using implanted electrodes. Recordings were 24/7.
Oil of White Widow was injected subcutaneously (20-50 mg/kg). Each rat received on 4 consecutive days an injection of this oil. According to the same schema, control rats received a similar volume of olive oil (Sigma Chemical Co., The Netherlands). As positive control the synthetic drug WIN 55, (Sigma Chemical Co., The Netherlands) was administrated once, subcutaneously (10 mg/kg).

Results:
Chronic administration of the White Widow oil did not influence the total time of immobile behavior. However, White Widow oil decreased the number of segments of immobile behavior, and vice versa, it increases the mean duration of these segments. Spectral analyses of the EEG revealed a global loss of power in the low (1-14 Hz) frequency bands.

Discussion:
Both cannabinoids, - the synthetic WIN55 and the phytocannabinoid White Widow oil - induced a defragmentation of the periods of immobility. Whether these periods indicate sleep or passive wakefulness remains to be analyzed. The observed drug-induced changes in brain wave activity conflicts with better sleep and might point to a pharmacological dissociation between EEG and overt behavior. Solving these questions might be critical when considering the therapeutic potential of cannabinoids in insomnia.

1 Schierenbeck T, et al.,. Sleep Med Rev. 2008, 12(5):381-389.
2 van Rijn, CM, et al., Epilepsia 2011, 51(8) 1511-1521.
[3) present affiliation: Leiden University, Leiden Institute for Brain and Cognition, The Netherlands]
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(14) PROPOFOL AT THERAPEUTIC DOSE IS NOT A HUMAN FATTY ACID AMIDE HYDROLASE INHIBITOR

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An animal study suggested that the narcotic drug propofol inhibits FAAH activity, at least in mouse brain membrane preparations. In humans, a previous study reported that total intravenous anesthesia (TIVA) with propofol lead to a stabilization of the plasma concentration of the endocannabinoid anandamide, presumably through FAAH inhibition, whereas with volatile narcotics, anandamide decreased. The mechanism could contribute to propofol´s pharmacological properties, namely decreased postoperative emesis. We hypothesized that during propofol-TIVA, plasma AEA is correlated to propofol blood concentrations, and that this correlation is explained by FAAH inhibition through propofol.
Methods: We studied 56 patients undergoing general anesthesia with either propofol or thiopental/sevoflurane for spinal surgery. Choice of the anesthetic protocol was at the discretion of the anaesthesiologist. Blood samples were obtained before anesthesia and 10min, 30min, and 60min after propofol or thiobarbital injection. AEA was measured by GC-MS/MS using deuterated standards, 2-AG was determined by LC-MS/MS. 2-way ANOVA for repeated measurements and AUCs for anandamide were calculated. FAAH activity was determined by incubation of biological samples or recombinant human FAAH with d_4-anandamide (d_4-AEA) as substrate and measurement of produced d_4-ethanolamine (d_4-EA).
Results: **1)** In both groups, plasma AEA concentrations rapidly decreased after anesthesia induction reaching a nadir at 10 min. At 30 min, AEA concentrations were still significantly decreased compared with baseline and then recovered towards baseline concentrations at 60 min. **2)** The response at each time point, and the corresponding area under the curve (AUC) for anandamide was similar with propofol and thiopental/sevoflurane. **3)** Plasma 2-AG concentrations were stable in the propofol group over 1 hour, whereas a small, but significant decrease occurred in the thiopental/sevoflurane group at 10 min. Nevertheless, **4)** 2-AG AUC over 60 min was similar in both anesthesia groups. **5)** Plasma concentrations of both endocannabinoids and propofol at 10, 30, and 60 min were not correlated with each other. **6)** d_4-EA formation in whole blood samples with narcotic concentrations of propofol or thiopental was identical to control samples without additives. In contrast, the potent FAAH inhibitor oloxa significantly inhibited d_4-EA formation. **7)** *In vitro* studies with recombinant human FAAH and d_4-AEA as substrate revealed identical enzyme kinetics with and without propofol.
Conclusions: We did not detect any effects of propofol on FAAH activity or AEA plasma concentrations in a sufficiently powered study employing validated analytical methods. Therefore, propofol's anesthetic and antiemetic actions in human subjects cannot be explained by peripheral FAAH inhibition. However, our findings do not exclude direct or indirect generated central cannabinoid receptor activation by propofol.

(15) ANTI-INFLAMMATORY EFFECT OF 5-AMINOSALICYLIC ACID ON THE PPARa SIGNALING SYSTEM IN THE HUMAN ULCERATIVE COLITIS

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Recent studies suggest potential anti-inflammatory roles of the peroxisome proliferators activated receptor alpha (PPARa) in inflammatory bowel diseases. Although PPARa receptor was decreased during experimental colitis and PPARa agonists-mediated anti-inflammatory activity was weakened in PPARa-null mice, the presence and function of PPARa signaling system in the ulcerative colitis of humans remain unknown.

Methods: Protein and gene expression of PPARa receptor and PPARa ligands-biosynthesis (NAPE-PLD) and -degrading (FAAH and ASAHL) enzymes were analyzed in both acute untreated active ulcerative colitis and treated quiescent patients in comparison with healthy human colonic tissue by immunohistochemistry and RT-PCR. Analysis was carried out according to clinical criteria of severity at onset and after the treatment with 5-aminosalicylic acid (5-ASA), corticosteroid or immunomodulators.

Results: Immunocytochemical and RT-PCR analyses indicated that PPARa, ASAHL, NAPE-PLD and FAAH were present in the colonic tissue, showing a differential distribution in the epithelium, lamina propria, smooth muscle and enteric plexus. Gene expression analysis showed a decrease of PPARa, PPARg and ASAHL and an increase of FAAH and iNOS in acute colitis. Quantification of epithelial immunoreactivity confirmed a decrease of PPARa, but showed an increase of ASAHL and a decrease of NAPE-PLD in acute colitis, which were partially restored to control levels after treatment. Costicosteroids and immunomodulators decreased significantly the effect of 5-ASA on PPARa expression. We also characterized the immune cells of the mucosa infiltrate during colitis. We detected a decrease in the number of ASAHL and an increase of FAAH-positive immune cells in acute colitis, which were partially restored to control levels after treatment.

Conclusions: 5-ASA signaling pathway, through PPARa, reduces colitis-associated inflammation suggesting PPARa agonists as potential drugs for the treatment of inflammatory bowel diseases in human.

(16) LIQUID EXTRACTION OF ENDOCANNABINOIDS WITH TOLUENE REDUCES MATRIX EFFECTS AND TRANS-ACYLATION OF 2-ARACHIDONOYL GLYCEROL TO 1-ARACHIDONOYL GLYCEROL

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Endocannabinoids are endogenous ligands of the cannabinoid receptors CB1 and CB2. Arachidonoyl ethanolamide (anandamide, AEA) and 2-arachidonoyl glycerol (2AG) are the two most studied endocannabinoids. Accurate measurement of both, AEA and 2AG, in various biological matrices even in the low nanomolar to high picomolar range play a key role in studies of the endocannabinoid system.. Application of triple quadrupole mass spectrometry has been shown to be a reliable way to accomplish endocannabinoid analysis, either in combination with gas chromatography (GC), or with liquid chromatography (LC). Some specific analytical difficulties are due to the lipid-structure of endocannabinoids. For example, they are hardly soluble in aqueous solutions and tend to stick to plastic surfaces. In addition, 2AG is subject to rapid isomerization to 1AG by spontaneous trans-acylation. Especially during sample preparation, trans-acylation may lead to wrong 2AG measurements. In order to monitor this effect, it is necessary to chromatographically separate 2AG and 1AG from each other since they share the same mass spectrometric properties

Methods: The LC-MS/MS system used consisted of a Waters Acquity UPLC pump, autosampler and column oven connected to a Waters XEVO TQ MS mass spectrometer. Chromatography was performed on a 60° C heated Waters Acquity BEH C18 column of 100 mm length, an inner diameter of 2.1 mm and a particle size of 1.7 µm. A gradient of water and methanol was used, both containing 2 mM ammonium acetate as modifier, starting at 25% water and 75% methanol changing to 10% water and 90% methanol over five minutes before switching back to initial conditions until the end of the run at 6.5 min. The mass spectrometer was used in the positive electrospray ionization mode with nitrogen at 600° C for the atmospheric pressure ionization gas and argon as collision gas. For AEA, the fragment ion at *m/z* 62 evolving from the precursor ion at *m/z* 348, for 2AG and 1AG the fragment ion at *m/z* 287 from *m/z* 379 was used for measurements. Quantitative measurements were accomplished by addition of deuterated internal standards, i.e. d_4-AEA (*m/z* 352 to *m/z* 66) and d_5-2AG (*m/z* 384 to *m/z* 287).

Results. The LC-MS/MS method is capable of 2AG/1AG separation. The use of toluene for liquid extraction provides two important benefits: **1)** Isomerization from 2AG to 1AG is minimized during the evaporation of toluene as compared to protic organic solvents such as methanol or ethanol. **2)** Toluene extracts contain less phospholipids that **3)** contribute to matrix effects that may disturb sensitive measurements.

Conclusions: In conclusion, the liquid extraction of endocannabinoids with toluene offers a quick and easily feasible sample preparation method that allows for sensitive and accurate LC-MS/MS measurements.

(17) SYSTEMATIC CHARACTERIZATION OF ENDOCANNABINOID MICRODIALYSIS

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In-vivo microdialysis of endocannabinoids has been performed over the last decade primarily in brain tissue of rodents. The lipid characteristics of anandamide (AEA) and 2-arachidonoyl-glycerol (2AG) impose a great challenge on the implied methods for microdialysate sample collection and analysis. Low solubility in aqueous media, adsorption to surfaces and instability with co-present human albumin are the obstacles to overcome in endocannabinoid microdialysis.

Methods: We characterized endocannabinoid microdialysis using an in-vitro microdialysis system. We employed Ringer's solution with 10% (w/v) hydroxypropyl-ß-cyclodextrine (HPCD) as perfusion fluid, Ringer's solution with human fatty acid free serum albumin (HSA) as matrix fluid, and CMA 20 kDa cut-off microdiaylysis cathethers with poly aryl ether sulphone (PAES) membrane. AEA and 2AG were extracted from microdialysates by liquid extraction using toluene and measured by LC-MS/MS. Experiments were performed using the recovery and delivery methodology and feasibility of calibration was evaluated by flow-rate variation. Additionally, the systems ability to map changing AEA concentrations in the matrix fluid was studied by alternating matrix fluid with 1 µM AEA or without AEA. In a proof-of-concept study in two human normoweight healthy humans, microdialysate from abdominal subcutaneous adipose tissue was collected and AEA concentrations measured by LC-MS/MS.

Results: **1)** AEA was found to be stable both in perfusion fluid and matrix fluid. **2)** 2AG was only stable in perfusion fluid, and underwent rapid isomerization to 1AG in matrix fluid.. **3)** AEA and 2AG could be microdialyzed with a relative recovery of 6.5% when 10% (w/v) HPCD and a perfusion fluid flow rate of 2 µL/min was used. However, since 2AG was found unstable in the matrix fluid, reliable calculation of relative recovery rates required matrix fluid 2AG concentrations measurements over time. **4)** Inward and outward endocannabinoid flow over the PAES membrane was found to be unequal. **5)** Contrary to usual findings in microdialysis studies, recovery rates for AEA increased with increasing flow rate to a maximum of 6.5% at 2 µL/min. **6)** Long equilibration times of several hours were found to be necessary in order to obtain constant relative recovery rates. **7)** Microdialysis of AEA from matrix fluid containing AEA concentrations in the low nanomolar range is possible when 10% HPCD was used in the perfusion fluid. Further requirements were a flow rate of 2 µL/min and a microdialysate sampling interval of 2 h. **8)** In a proof of concept study we found that AEA can be microdialysed from abdominal adipose tissue and measured by LC-MS/MS at a concentration of 102 and 38 pM.

Conclusions: Endocannabinoid microdialysis is possible with low relative recoveries. However, determination of absolute tissue concentrations by calibration through flow-rate variation is impossible. HPCD is required in the perfusion fluid and highly sensitive analytical devices are needed for the reliable measurement of endocannabinoid concentrations in microdialysate samples.

(18) DESIGN AND SYNTHESIS OF NEW QUINOLONE-7-SUBSTITUTED-3-CARBOXAMIDES AS POTENTIAL CB1 SELECTIVE LIGANDS

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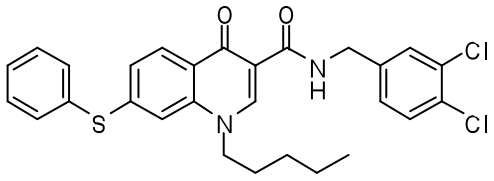
A lot of progress has been achieved in the last years in understanding the action mechanism of cannabinoids. The identification of two different receptor types (CB1, CB2) which are differently located in the human organism and can be selectively inhibited, has revived the therapeutic interest in these substances. On the basis of the structure-selectivity relationship developed in our previous work (*Pasquini et al., J. Med. Chem. 2008, 51: 5075-5084*), we decided to investigate the effect of new quinolonos on cannabinoid receptor affinity/selectivity, systematically changing the substituents on C3, N1 and C7 position. We designed and synthesized quinolones bearing at C7 diverse aryl substituents. Then we introduced different substituents on the carboxamide nitrogen, maintaining the 7-phenylthio group. Finally, we introduced different alkyl chains at N1 position on compound **1**.

Methods: The target compounds bearing aryl groups at N1 were synthesized according to a modified Grohe-Heitzer procedure (*Cecchetti et al., J. Med. Chem., 1996, 39:436-445*): treatment of 2,4-difluorobenzoyl chloride with 3-dimethylaminopropenoic acid ethyl ester led to the enamino ketones that were reacted with iodo-alkyl reagents. Esters so obtained were hydrolyzed and transformed into amides by coupling with the appropriate amine, affording the final compounds after substitution of fluorine atom with thiophenol. In a different synthetic pathway, intermediate esters obtained according to Gould-Jacobs and Lappin cyclization reactions from the appropriate anilines were first *N*-alkylated, substituted at C7 position with thiophenol then hydrolyzed to the corresponding acids and converted into final amides.

Results: 1) Most of the tested compounds have shown high affinity for both CB1 and CB2 receptors; 2) with the most lipophilic chains, very potent though poorly selective ligands were obtained; 3) aryl groups at C1 position caused the loss of receptor affinity; 4) substituents on the amide nitrogen had a more marked effect on CB2 affinity than on CB1 affinity.

Conclusion: The substitution at C7 position with a phenylthio group seemed necessary for a good CB1 affinity. Compounds with reverse selectivity were identified: in particular, **1** is completely selective CB1.



1 *Ki* CB1 = 420 nM,
Ki CB2 = > 10000 nM
Si = 0.042

(19) IMMUNOAFFINITY CHARACTERIZATION OF PEPTIDE ENDOCANNABINOIDS IDENTIFICATION OF N-TERMINAL EXTENDED PRECURSORS

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The α-hemoglobin derived peptides RVDPVNFKLLSH and VDPVNFKLLSH were reported to be potential endogenous peptide ligands for the cannabinoid receptor CB1 based on MS/MS experiments (Gomes et al., FASEB J. 2009, 23(9):3020-9). However, so far no absolute quantification was possible as a suitable mAb was missing.

Methods: To study the distribution and quantity of a putative CB receptor binding end-product of this peptide family we have raised monoclonal antibodies (mAb) against the C-terminal part of RVD-Hpα. A suitable high-affinity mAb enabled us to identify RVD-Hpα and related peptides using immunoaffinity MS/MS experiments and Western blots as well as their quantification mouse brain and in human plasma by using competitive ELISA.

Results: mAbs were generated and their affinity, specificity and epitopes were characterized. N-terminal extended precursors were identified in mouse brain extracts, human and mouse plasma. Importantly, the shorter forms hemopressin (PVNFKLLSH; Hpα) and VD-Hpα were not detected, thus providing further evidence that they are extraction artefacts. Therefore, we propose the name peptide endocannabinoids 12-23 (Pepcans 12-23). In radioligand displacement experiments we detected binding of Pepcans to a CB_1 receptor binding site different from CP55,940. The CB_1 receptor binding affinity decreased with N-terminal chain length.

Conclusions: We have established a method to successfully quantify the peptide endocannabinoids from tissues. Pepcans-12, -17 and 20 are abundant in brain, but also plasma. The previously suggested CP55,940 competitive binding (endocannabinoid binding site) interaction of hemopressin and Pepcans with the CB1 receptor was not confirmed, but a different CB1 binding site is postulated.

(20a) SYNTHESIS OF 1,3,7-TRISUBSTITUTED XANTHINE DERIVATIVES AS LIGANDS FOR CANNABINOID AND RELATED GPCRS

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The natural xanthines caffeine and theophylline and their pharmacological effects in humans and animals are well known. The xanthine scaffold can be envisaged as a privileged structure in medicinal chemistry since xanthine derivatives which selectively interact with different receptors or enzymes, e.g. adenosine receptors, or phosphodiesterases, respectively, have been described. The bicyclic xanthine core allows for the easy attachment of a variety of substituents in different positions, in particular at the ring nitrogen atoms *N1*, *N3* and *N7*.
In the present study we investigated whether the xanthine scaffold could be used to prepare ligands for cannabinoid (CB) receptors and the related orphan receptor GPR55 by substitution with lipophilic substituents based on known pharmacophore models for CB receptors.
Methods: For the introduction of a large spectrum of *N*1-substituents, 3,7-disubstituted xanthines were prepared as precursors starting with six differently substituted amines (providing the final *N*3-substituent) to build up the xanthine heterocycle. Alkyl and arylalkyl side-chains were introduced at *N*1 by standard alkylation procedures under basic conditions. Further *N*3-variation was achieved by alkylation of paraxanthine analogs, which were obtained by cleavage of *N*1-benzylxanthine derivatives.
A variety of substituents at *N*7 could be obtained by microwave reaction of 5-bromouracils with the corresponding amines.
Results: **1)** A small library of so far about 50 compounds was synthesized. **2)** First results of the investigation at human CB receptors and the GPR55 are promising with regard to receptor affinity and selectivity (see for test results and further data on neighbouring poster by Viktor Rempel et al.).
Conclusions: **a)** Xanthines constitute a privileged structure in medicinal chemistry and offer possibilities for broad structural variation. **b)** 1,3,7-trisubstituted xanthines exhibit a novel scaffold for the development of new potent and selective ligands for both CB and GPR55 receptors.

(20b) XANTHINE DERIVATIVES AS NOVEL LIGANDS FOR CANNABINOID RECEPORS AND THE RELATED ORPHAN RECEPTOR GPR55

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The diverse physiological effects of D^9-tetrahydrocannabinol (THC), the main bioactive constituent of the C*annabis sativa* plant, are mediated through the activation of cannabinoid (CB_1 and CB_2) G protein-coupled receptors (GPCR). While the CB_1 receptor is predominantly expressed in the CNS, the CB_2 receptor can primarily be found on immune cells. Recently, cannabinoids as well as the CB_1 receptor inverse agonist rimonabant were found to activate the orphan GPR55 at micromolar concentrations, raising the question whether the GPR55 receptor might be a third cannabinoid receptor subtype. However, only very little is known so far about GPR55 and its ligands. Therefore potent ligands are required to elucidate the physiological role of GPR55 and its potential as a novel drug target. We have designed, synthesized and pharmacologically characterized a series of xanthine derivatives as CB and GPR55 receptor ligands, and were successful in obtaining CB_2 receptor selective agonists and GPR55 antagonists.

Methods: Compounds were tested for affinity in heterologous radioligand binding experiments at both human CB receptor subtypes. Compounds showing a K_i value of <1 µM were funcionally characterized in cAMP accumulation assays. Interaction with the GPR55 receptor was investigated by measuring β-arrestin translocation to the activated receptor, which was detected by measuring luminescence emission, based on β-galactosidase enzyme fragment complementation technology (β-arrestin PathHunter™ assay, DiscoverX).

Results: **1)** Some of the synthesized compounds show high CB_2 receptor affinity and selectivity. **2)** Bulky aliphatic residues in position 1 of the xanthine scaffold are preferred over aromatic or short aliphatic moieties. **3)** The most potent compound so far possesses affinity in the nanomolar range (K_i: 357 nM). **4)** Further pharmacological investigation revealed agonistic properties in cAMP accumulation assays. **5)** First results indicate that N1-benzyl substituted compounds possess antagonistic properties at GPR55.

Conclusions: **a)** The xanthine scaffold – a privileged structure in medicinal chemistry - is suitable for the development of CB and GPR55 receptor ligands. **b)** Novel CB_2-selective ligands with agonistic properties could be obtained. **c)** Antagonists at GPR55 could be identified which show selectivity versus CB receptors. **d)** The identified compounds provide a basis for the development of more potent and selective ligands at CB receptors and the GPR55.

(21) DEVELOPMENT OF POTENT ENDOCANNABINOID REUPTAKE INHIBITORS FROM A PLANT DERIVED *N*-ISOBUTYLAMIDE SCAFFOLD

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Plant fatty acid derivatives such as *N*-acylethanolamines and polyketide *N*-alkylamides are structurally related to endocannabinoids. Certain C12 *N*-isobutylamides from purple coneflower (*Echinacea* spp.) have been shown to interact with the CB2 receptor. Some of these *N*-alkylamides also weakly (partially) affect the reuptake of anandamide (AEA) into cells. We have synthesized a library of compounds based on the natural 2E4E-dodecadiene scaffold and screened them on different targets within the endocannabinoids system. Several *N*-alkylamides showed a potent inhibition of AEA reuptake.

Methods: Amides were synthesized using A Horner-Wadsworth-Emmons reaction between commercially available aldehydes and triethyl phosphonoacetate with NaH in THF gave the unsaturated esters. After saponification with NaOH in MeOH, treatment of the resulting acids with thionyl chloride provided the corresponding acid chlorides, which were further transformed into the desired amides by reations with the appropriate amines. Reuptake was measured using [3H]-AEA and GC/MS in U937 cells. FAAH assays were performed with cellular homogenates and recombinant hFAAH.

Results: Different natural and non-natural *N*-alkylamides were screened for modulatory effects on the cellular endocannabinoid system (ECS). Guineensin from *Piper longum L.*was found to inhibit the transport with an EC_{50} value in the nM range. By modification of the isobutylamide head group and conserving the 2*E*4*E*-dodecadiene chain novel lipids were obtained that showed selective nanomolar AEA reuptake inhibition. The *N*-3-methoxybenzylamide strongly and selectively inhibited uptake of AEA into U937 cells without modulating FAAH activity. Moreover, new insights into the SARs of *N*-alkylamides with ECS targets could be obtained.

Conclusions: We show that *N*-alkylamides other than ethanolamides and independent of an arachidonoyl tail can influence anandamide uptake into mammalian cells. Based on the natural *N*-alkylamide scaffold we developed highly potent AEA uptake inhibitors which could serve as lead compounds. The natural product guineensin is shown for the first time to be a potent AEA uptake inhibitor. Overall, FAAH inactive AEA reuptake inhibitors will be crucial for the study of the endocannabinoid transport system and may therefore serve as tools to better understand lipid transport within the ECS.

(22) THE C385A MISSENSE VARIANT MODULATES ACUTE STRESS RESPONSE IN HEALTHY HUMANS

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Introduction: Recent evidence has shown that the effects of stress are modulated by endogenous cannabinoids, which act as agonists at the cannabinoid receptor 1 (CNR1) and are degraded by the enzyme fatty acid amide hydrolase (FAAH). In this study, we investigated whether individual differences in mood response to an acute social stressor were related to the rs1049353 CNR1 gene variant or the functional rs324420 (Pro129Thr) FAAH gene polymorphism, that was previously found to be associated with street drug use, addictive traits, amphetamine response, anorexia nervosa and depression.

Methods: Caucasian healthy adults (N=73) participated in two sessions involving either a standardized psychosocial stress procedure (Trier Social Stress Test) or a control task. Subjective (Profile of Mood States, POMS) and physiological measures were obtained before and at regular intervals after the tasks. Associations between individual genotypes and levels of self-reported Anxiety and Confusion (POMS) after stress exposure were investigated using two-way ANOVAs/ANCOVAs.

Results: The functional rs324420 (Pro129Thr) FAAH gene variant was significantly associated with increases in Anxiety ($p=0.017$) and Confusion ($p=0.00019$) after stress. The association between rs324420 and increases in Confusion remained significant after adjustment for mulitple testing. There was no association between the rs1049353 CNR1 variant and mood response after stress.

Conclusions: These results support the idea that the human endogenous cannabinoid system is significantly involved in stress response. Genetic variability in the FAAH gene may modulate perception of stress and thus modulate the individual´s risk for developing a stress-related psychiatric disorder. This finding suggests that manipulations of the cannabinoid system may offer strategies for prevention and treatment of mental illness.

(23) A MOUSE LINE FOR CELL TYPE-SPECIFIC RESCUE FROM CB1 RECEPTOR DEFICIENCY

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In the brain, the endocannabinoid system is involved in the regulation of a large variety of functions, including neuroprotection, feeding behavior stress responses, anxiety and extinction of fear memories. To further understand the mechanism underlying these processes, we generated a novel mouse line for cell type-specific rescue from CB1 receptor deficiency (Stop-CB1). A loxP-flanked transcriptional stop cassette in the CB1 receptor gene locus represses CB1 receptor expression throughout the entire body, including the brain.
By crossing this mouse line to a mouse line ubiquitously expressing Cre recombinase (*Ella-Cre*), we demonstrated the reactivation of CB1 receptor expression by excision of the stop cassette. The complete reactivation of CB1 receptor expression was confirmed by histological analysis (Western Blot and immunohistology) and in vivo experiments, i.e. kainic acid induced seizures and acute food intake after starvation.
To further address the role of intact CB1 receptor signalling in specific neuronal subpopulations, we crossed the Stop-CB1 line with transgenic mouse lines that express Cre recombinase selectively in glutamatergic (Nex-Cre) or GABAergic (Dlx5/6-Cre) neurons. Thus, endogenous levels of CB1 receptor are reactivated locally and we will be able to investigate if expression of CB1 receptor in a specific cell population is sufficient to rescue the deficiencies observed when CB1 receptor signalling is missing.

(24) GENERATION OF CB2 RECEPTOR HUMANIZED MICE

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Introduction: Mammalian tissues contain two types of cannabinoid receptor, CB1 and CB2, which belong to the family of G protein coupled receptors. Endocannabinoid binding to these receptors activates several cellular signalling pathways including the inhibition of the adenylyl cyclase–cyclic AMP–protein kinase A activity, activation of mitogen-activated protein kinase cascades (extracellular-signal-regulated kinase (ERK), JUN amino-terminal kinase (JNK) and p38); and activation of the phosphatidylinositol-3-kinase–AKT pathway. In general CB1 receptors are expressed at high levels in the central nervous system, whereas CB2 receptors are found predominantly in peripheral tissues e.g. on bone and immune cells. Our team has recently identified two different variants of the human CB2 receptor hCB2Gln63 and hCB2Arg63, respectively. Interestingly, the hCB2Arg63 variant has been associated with reduced bone density, osteoporosis, and psychiatric disorders in different human population samples. These findings are in concordance with the demonstration of a reduced signaling of the hCB2Arg63 variant.

Objectives: To study the functional consequences of these gene variants *in vivo*, we are generating humanized mice harboring both CB2 receptor variants. These mice will be a useful tool to study the *in vivo* effects of selective CB2 agonists and CB2 pathomechanisms in pain, osteoporosis and immune diseases. In addition these mice could be helpful in development of new drug, which will more precisely target the human CB2 receptor.

(25) GENERATION OF CONDITIONAL KNOCK OUT MICE FOR DIACYLGLYCEROL LIPASE A AND B

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Introduction: The endocannabinoid system (ECS) is a retrograde signaling system that plays an important role in neuroprotection and synaptic communication throughout the nervous system. As retrograde messenger molecules, the endocannabinoids 2-arachidonoyl glycerol (2-AG) and anandamide (AEA) act at a wide variety of different synaptic circuits. The main endocannabinoid 2-AG is produced on demand from diaglycerol precursor molecules by postsynaptically located diacylglycerol lipases (DAGL). Released into the synaptic cleft, 2-AG can bind and activate presynaptic, G-protein coupled cannabinoid receptors, which in turn reduce the transmitter release from the presynaptic neuron. Due to the fact that anandamide as well is capable of activating cannabinoid receptors on the presynaptic side of the signaling machinery, it is challenging to credit certain physiological effects of the ECS to a certain messenger. To address this problem we chose to selectively switch off the production of 2-AG in a knock out approach. Therefore we are generating conditional knock out mice for the two isoforms of the 2-AG producing enzymes DAGL a and b.

Methods/Results: For the construction of conditional knock out targeting vectors, the Red®/ET® cloning system developed by genebridges™ was used. The application of this system exploits the advantages of homologous recombination in *E. coli* and circumvents the dependence on restriction enzymes. The linearized constructs were electroporated into different ES cell lines (129sv and C57Bl/6). Chimeric mice were generated for both lines by blastocyst injection. Up to date germline transmission has been successful for the DAGL a construct on the 129sv as well as on the C57Bl/6 background.

Conclusions: The different knock out lines will facilitate the detailed analysis of the physiological roles of 2-AG in the endocannabinoid system and simplify the differentiation between 2-AG mediated effects and the effects of other endocannabinoids. The conditional approach of the knock out will allow a tissue- and area specific elimination of 2-AG. This enables the creation of a detailed map of 2-AG function in the central nervous system as well as in peripheral tissues.

(26) EVIDENCE SUPPORTING THE EXISTENCE OF AN ENDOCANNABINOID MEMBRANE TRANSPORTER INVOLVED IN CELLULAR AEA AND 2-AG UPTAKE

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Endocannabinoids (ECs) are key mediators involved in many physiological and pathological conditions in CNS and peripheral tissues where they exert biological activities by interacting with extracellular and intracellular targets. The effects of ECs are regulated by cellular biosynthesis, release, re-uptake, trafficking and enzymatic metabolism. Unlike the clear knowledge about the biosynthetic and metabolic pathways of ECs, their cellular re-uptake is still a debated issue with several mechanisms proposed. One of the principal issues in elucidating this mechanism is the tight inter-play between ECs plasma membrane movement and their rapid and almost complete cellular cytoplasmic cleavage by the enzymes FAAH and MGL.

Methods: Radioactivity assays, TLC analysis and analytical (GC/MS) quantification of ECs in whole cells (U937 human monocytic cell line) was developed to investigate the intracellular and extracellular level of AEA, 2-AG and their degradation products. This new combined approach has been applied to investigate the uptake and hydrolysis of AEA and 2-AG when co-incubated or when incubated alone in presence of specific endocannabinoid membrane transporter (EMT) inhibitors, enzymatic inhibitors (FAAH, MGL) and combinations of them.

Results: **1)** Radioactivity-based results show additive and super-additive effects in AEA uptake inhibition and AEA extracellular accumulation when combining an EMT inhibitor with a FAAH inhibitor in comparison to the effect of the FAAH inhibitor alone **2)** GC/MS quantification showed an increase of AEA level intracellular following FAAH inhibitor treatment while EMT inhibitor and the combination determined a reduction **3)** TLC analysis of the intracellular radioactivity showed that the vast majority of the signal derives from [^{3}H]-ethanolamine incorporated into phospholipids and not from intact [^{3}H]-AEA **4)** EMT inhibitors were tested in cells expressing low- or high-level of fatty acid binding proteins (FABPs, recently identified as AEA intracellular carrier proteins) showing no difference in AEA uptake inhibition potency, while the highly FABPs-expressing cells were much more sensitive to a selective FABPs-inhibitor **5)** Co-incubation assays with AEA and 2-AG showed that these molecules compete for the same membrane transporter and that it is more selective towards AEA **6)** The MGL inhibitor JZL184 induced an intracellular accumulation of 2-AG, while EMT inhibitors reduced the intracellular level in both radioactivity assays and GC/MS quantification **7)** TLC analysis show no incorporation of [^{3}H]-glycerol into phospholipids.

Conclusions: The results obtained by using radioactivity assays, GC/MS quantification and TLC analysis in presence of different FAAH-, MGL- and EMT inhibitors alone or in combination provide evidence in favor of the presence of a common EMT involved in AEA and 2-AG cellular uptake. Our data indicate that AEA and 2-AG compete for the same membrane transporter (EMT) when co-incubated and that the transporter possesses a higher selectivity towards AEA.

(27) AMINO ACIDS D2.63 AND K373 ARE IMPORTANT FOR MAINTAINING THE CB1R BINDING POCKET, WHILE RESIDUES K3.28 AND S1.39 ARE INVOLVED IN SELECTIVE LIGAND RECOGNITION

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Previous work has demonstrated that specific amino acid residues in G-protein coupled receptors (GPCRs) are important for receptor stimulation and ligand recognition. In the absence of crystal structures for the CB_1 or CB_2 proteins, much of the structural information on these proteins has been gained from biochemical, mutational, and modeling studies. D2.63 is a unique aspartate that is at the extracellular end of TMH2, facing into the binding pocket of CB1. We have previously demonstrated that mutation of D2.63 to N does not affect ligand binding but does play a crucial role in modulating signal transduction (Kapur et al, 2008). K373 is located on the 3rd EC loop of the CB1 receptor. Together, D2.63 and K373 are thought to form a salt bridge under normal receptor activation. The 3rd transmembrane domain contains a highly conserved lysine residue at position K3.28 (K192). Previously, an alanine substitution of K3.28 (K3.28A) showed that only WIN55,212 (WIN) could maintain CB_1 receptor binding (Song and Bonner, 1996; Chin et al, 1998). CP55,940 (CP) binding was eliminated, as was that for HU210 and anandamide. These and subsequent studies suggest that specific residues are important for recognizing different ligands, while other residues may be important for maintaining the binding pocket. We have also created a novel mutation in the first transmembrane domain of CB1 at S1.39. We generated alanine substitutions in the CB1 receptor by site directed mutagenesis, with stable expression in HEK293 cells. We used [^{35}S]GTPγS binding to measure the stimulation of WT and mutant cannabinoid receptors with distinct ligands. In the D.2.63A mutant, each cannabinoid that was tested generated a lower E_{max} and increased EC_{50} compared to WT. The values became increasingly significant when the 'salt-bridge' was completely disrupted at the D2.63A-K373A mutant. The EC_{50} increased greatly for JWH-018 (43 fold) in the D2.63A-K373A mutant receptor, while a 2 fold increased was seen at the D2.63A. Likewise, the E_{max}s for D2.63A and D2.63A-K373A mutants for JWH-018 were 88.7% and 59.2%. E_{max}s for CP stimulation at the D2.63A and D2.63A-K373A mutants were 73.4% and 28.9% and the EC_{50}s were 5.7 and 3.1 fold higher, respectively. The D2.63A-K373A mutant showed a severe impairment of receptor stimulation, representing a disruption of the 'salt-bridge' that is involved with maintaining the structure and functionality of the CB1 receptor.
K3.28A and S1.39A may be residues which are important for selective ligand recognition. While the values generated for the K3.28Aand S1.39A mutants by WIN stimulation where not significantly different from WT. At the K3.28A mutant but not the S1.39A, the indole JWH-018 was able to stimulate GTPγS binding similar to WT. The existence of amino acid residues in the CB1 receptor involved in selective ligand recognition is supported by our findings.

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(28) DEFINITION OF THE CELLULAR SOURCE OF ELEVATED 2-AG LEVELS IN THE LIVER AND ANALYSIS OF 2-AG INDUCED CELL DEATH IN HEPATIC STELLATE CELLS

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Introduction: Liver fibrosis is the response to chronic hepatic injury and results from an increased deposition of connective scar tissue by activated hepatic stellate cells (HSCs). It was recently shown that the endocannabinoid system plays an important role in liver fibrogenesis. In animal models of liver injury and fibrosis, the endocannabinoid 2-arachidonoyl glycerol (2-AG) becomes highly upregulated. Moreover, 2-AG specifically induces apoptosis in HSCs, the main fibrogenic cell type in the liver, but not in hepatocytes. Therefore, 2-AG exhibts anti-fibrotic potential. However, celluar origin and mechanisms that lead to increased hepatic 2-AG levels are unknown so far. Moreover, factors for the different susceptibility of HSCs and hepatocytes to 2-AG remain to be determined. A possible role of cyclooxygenase (COX)-2 that converts 2-AG to prostaglandin-glycerolester (PG-GE) was taken into account.

Methods: Primary hepatocytes, HSCs, liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) from healthy, murine livers were isolated by collagenase perfusion and MACS, respectively. Diacylglycerol lipase (DAGL) α and β, fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MGL) and COX-2 mRNA levels were determined by quantitative realtime PCR or western blot in livers of healthy or bile duct ligated (BDL) mice. 2-AG-, PGD2-GE, PGE2-GE or PGF2α-GE-induced cell death was analyzed by LDH assay and western blots for caspase 3- and PARP-cleavage were performed as a marker for apoptotic cell death. PGD2-GE production was measured by PGD2-GE-MOX-ELISA.

Results: The results showed a fivefold upregulation of the expression of the main 2-AG-producing enzyme DAGLβ 14 days after BDL whereas the expression of the degrading enzymes FAAH and MGL decreased significantly seven days after BDL. Interestingly, DAGLβ was expressed in the non-parenchymal cells of the liver and was highest in HSCs and LSECs. The expression of DAGLβ in the liver is much stronger compared to DAGLα on cellular levels as well as in whole liver tissue. Starting seven days after surgery, a significant decline in FAAH as well as in MGL were observed in livers of BDL mice. On the cellular level, HSCs and LSECs displayed the highest mRNA levels of MGL. In contrast to MGL, FAAH is mainly expressed in hepatocytes. HSCs displayed significant COX-2 m-RNA expression, whereas hepatocytes did not express notable levels of COX-2. Similarly to 2-AG, PGD2-GE also induced apoptotic cell death dose-dependently in primary HSCs. However, PGE2- and PGF2α-GE did not induce apoptosis in HSCs. Primary HSCs, but not hepatocytes were able to metabolize 2-AG to PGD2-GE.

Conclusion: Elevated 2-AG levels during liver fibrogenesis are due to elevated expression of the synthesizing enzyme DAGLβ and decreased levels of the degradation enzymes FAAH and MGL. The data further point to non-parenchymal cells rather than hepatocytes being the major cellular source of 2-AG in the liver, whereas hepatocytes mainly contribute to 2-AG degradation. HSCs and hepatocytes differentially express COX-2, leading to differential metabolization of 2-AG. The generation of pro-apoptotic PGD2-GE by COX-2 in HSCs possibly contributes to the different susceptibility of hepatocytes and HSCs towards 2-AG-induced cell death.

(29) REGULATION OF TYPE 1 CANNABINOID RECEPTORS AND MU OPIOID RECEPTORS BY MICRORNA AND POSSIBLE CONSEQUENCES ON DRUG TOLERANCE

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Effects of cannabinoids are mediated mainly by the cannabinoid receptors type 1 (CB1) and type 2. CB1 receptors are predominantly and with a high abundance expressed in the central and peripheral nervous system. Effects of most of the clinically used opioids like morphine are mediated by mu opioid receptors (MOR), which are expressed in distinct neuronal cells. CB1 and MOR belong to the family of Gi/Go-protein-coupled receptors. Furthermore, they are often coexpressed in cells of the nervous system including human neuroblastoma SH SY5Y cells and primary striatal neurons from rats.
In the last decade it has been found that small RNA species termed microRNAs regulate genes at the posttranscriptional level. MicroRNAs bind specificly to a gene's mRNA resulting in translational repression and gene silencing.
Here, we investigated the effects of microRNAs on the expression and functional efficacy of CB1 and MOR in SH SY5Y cells and primary striatal neurons from rats. In addition, effects of chronic treatment of the cells with morphine and methanandamide on the expression of CB1 and MOR were studied, and a possible involvement of microRNAs in the receptor downregulation produced by chronic drug treatment was investigated.

Methods: Human neuroblastoma cells SH SY5Y were transiently transfected with plasmids resulting in overexpression of the microRNAs let7A, let7D, mir23B and mir98. CB1 and MOR function was assessed by (I) determining the phosphorylation of p42/44 MAP kinase induced by morphine and methanandamide, (II) determining the decrease in the intracellular cAMP content induced by morphine and methanandamide, and (III) radioligand binding assays. Morphine was used in a concentration of 1 µM and methanandamide was used in a concentration of 500 nM. Anti-hsa-let-7d (Qiagen, Hilden, Germany) was used as an inhibitor of let7D.

Results: **1)** Overexpression of let7D and mir23B in SH SY5Y cells resulted in decreased CB1 activity, while let7A and mir98 had no effect. **2)** Overexpression of let7A, let7D and mir98 in SH SY5Y cells resulted in decreased MOR activity, while mir23B had no effect. **3)** Chronic morphine or methanandamide treatment of primary striatal neurons from rats produced a functional down-regulation of both receptors resulting in cross-tolerance to morphine and methanandamide. **4)** An inhibitor of let7D significantly reduced this cross-tolerance.

Conclusions: These findings indicate, that the expression of CB1 and MOR is regulated by distinct microRNAs, e. g. let7D. Our experiments with a specific inhibitor of let7D, together with recent observations demonstrating an induction of let7 microRNA species in response to morphine (*He et al., J. Neurosci. 2010; 30: 10251-10258*), give reasons to hypothesize that regulation of CB1 and MOR by microRNA is involved in drug tolerance and cross-tolerance.

(30) ACUTE AND CHRONIC CANNABINOID TREATMENT DIFFERENTLY AFFECT ALCOHOL INTAKE IN PUBERTAL AND ADULT RATS

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Adolescence represents a period of increased vulnerability to the initiation of drug use and abuse. Cannabis and alcohol are among the most widely used drugs in youth, and are often co-consumed. The early onset of drug use might consequently lead to severe addictive disorders and drug abuse problems in later life. The present study assessed the acute, chronic and lasting effects of pubertal and adult cannabinoid treatment on repeated alcohol intake and on alcohol reward in male Wistar rats. Therefore, a pubertal (pd40) and an adult group of rats was either treated with the synthetic cannabinoid agonist WIN 55,212 (WIN) or vehicle. Acute administration of WIN was found to exclusively stimulate initial alcohol intake in pubertal rats, whereas no effects were observed in adult rats. A subsequent chronic WIN treatment with daily injections stimulated alcohol intake in pubertal and adult rats, but was more pronounced for the pubertal treatment. After an abstinence phase, the lasting effects of pubertal and adult WIN treatment were evaluated in adult rats with limited-access intake and progressive ratio testing for alcohol. Whereas no lasting WIN effect could be found for adult-treated rats, pubertal cannabinoid treatment promoted consumption and the reinforcing effects of ethanol in adulthood. Additionally, alcohol exposure during puberty was found to increase adult sensitivity for alcohol reward. These data clearly demonstrate a higher vulnerability for the acute and chronic effects of cannabinoids during puberty on alcohol intake. Additionally, pubertal but not adult cannabinoid treatment affected the rewarding properties of ethanol persistently.

(31) INVOLVEMENT OF THE ECB SYSTEM IN HEDONIC REWARD PROCESSING– MEASURED BY THE PLEASURE ATTENUATED STARTLE PARADIGM

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Introduction: Reward-related behaviors are very complex and the term "reward" includes a variety of different connotations that are mainly linked to appetitive emotions, the hedonic impact and reward motivation, but also include learning and extinction processes. Along with the dopaminergic and the endogenous opioid system, the endocannabinoid (ECB) system has emerged recently as a key neurochemical mediator for reward processing. It is well known that cannabinoids affect learning processes and can induce reinforcing and rewarding effects in humans and animals and growing evidence indicates that the ECB system modulates various aspects of drug and non-drug reward. However, the involvement of the ECB system in hedonic aspects of reward is not completely understood so far.

Methods: With the present study we investigated the modulatory role of the ECB system on hedonic perception, measured by the pleasure attenuated startle (PAS) paradigm. Here, the acoustic startle response (ASR) is reduced in the presence of a conditioned stimulus which has been previously paired with a reward. This cue-induced reduction in ASR is not related to attentional alterations or a more general arousal by odor presentation, the conditioned olfactory cue rather elicits a pleasant emotional state during which the ASR is inhibited. PAS was measured after either pharmacological (injection of the CB1 receptor antagonist SR141716) or genetic modulations in ECB signaling (strain differences in CB1 expression and CB1 receptor knockout mice).

Results: Pharmacological inhibition, attenuated activity of ECB signaling and the absence of CB1 receptors was found to reduce PAS without affecting the ASR amplitude. Furthermore, intake of a palatable food reward was also reduced under all conditions tested.

Conclusion: These data indicate that the ECB system, beside its important role for motivation and reward learning, appears to be also highly important for the mediation of hedonic aspects of reward processing.

(32) CANNABIDIOL ATTENUATES SOCIAL ISOLATION-INDUCED AGGRESSION IN MICE

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Cannabidiol (CBD) is a non-psychotomimetic compound from *Cannabis sativa* plant that induces anxiolytic- and antidepressant-like effects in rodents after systemic administration. Long-term individual housing increases aggressive behavior in mice, a condition termed isolation-induced aggression or territorial aggression, which can be attenuated by some anxiolytic and antidepressant drugs. Therefore, the aim of the present study was to verify if CBD could also modulate the aggressive behavior induced by social isolation.
Methods: Male C57BL/6J mice (8–10 weeks of age when test began) were housed individually in cages (24x17x12 cm) while mice conspecific were housed under normal conditions (five per gage). After 4 weeks of social isolation, we tested whether acute treatment with CBD (15, 30 and 60 mg/kg; n=6-7/group), administered intraperitoneally 30 min prior to test, would inhibit aggressive behavior in the intruder-evoked aggressive test. In this model, an intruder mouse of the same gender was placed in a resident home cage. The resident-intruder interactions were videotaped for 10 min and the latency to first bite against the intruder, the number of attacks and total duration of aggressive encounters were recorded.
Results: CBD (at doses of 15, 30 and 60 mg/kg) inhibited initiation of aggressive behavior, indicated by greater latencies to attack ($F_{3,22}$=5,75; P<0,01). Moreover, CBD also was able to reduce the number of attacks ($F_{3,22}$=4,51; P<0,05) and duration of aggressive behavior ($F_{3,22}$=11,99; P<0,001).
Conclusions: These findings suggest that CBD produces anti-aggressive effects and may be a useful drug for inhibit heightened aggressiveness, and possibly to treat aggressive behavior associated with psychiatric disorders.

(33) DIAGNOSIS OF MAJOR DEPRESSION USING ETHANOLAMINE PHOSPHATE (EAP): POSSIBLE APPLICATION FOR DEVELOPMENT OF ANTIDEPRESSANT MANIPULATING THE ENDOCANNABINOID SYSTEM

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The diagnosis of psychiatric disorders are still based solely on oral interview. Molecular markers which could improve the current classification of psychiatry disorders and stratify patients on a molecular biological basis into more homogeneous subgroups, are needed. In order to identify novel markers for major depression, we have applied a comprehensive profiling of metabolites using plasma samples.

Methods: Plasma levels of 538 metabolites were assayed by high-throughput mass spectrometry and statistically compared by Mann-Whitney test between 34 subjects with major depression and 38 matched controls. All diagnoses were done based on Structured Clinical Interview for DSM-IV Axis I Disorders. The Center for Epidemiologic Studies Depression Scale (CES-D) was used to assess severity of depression. Independent validation subjects including those from periphery disease groups were recruited in the same way and the total number of investigated subjects in this study reached 97. Spearman's correlation between metabolites was used to infer mechanisms of biomarker alteration. Statistical tests were performed using $\alpha = .05$, with the Bonferroni correction for multiple test when appropriate.

Results: Thirteen metabolites were identified as differentially expressed in major depression. The differential metabolites included neurotransmitter and monoamine metabolites which association with depression has previously been reported. Newly discovered biomarker was ethanolamine phosphate (EAP) which was significantly ($P < 10^{-9}$) lower for MDD subjects (1.83 ± 0.80 μM) than for controls (3.96 ± 2.95 μM). Of note, the plasma levels of EAP alone was sufficient to distinguish MDD subjects from non-MDD subjects (true positive rate=82%, true negative rate=95%). The plasma EAP level was negatively correlated with CES-D depression score ($r = -0.433$). The EAP alteration were validated in 11 independent validation subjects. The decrease in plasma EAP level disappeared after successful antidepressant treatments. Among 538 metabolites, taurine showed very strong positive correlation with EAP. Both EAP and taurine are hydrolysate of endocannabinoid and target of fatty acid amide hydrolase (FAAH) that terminates endocannabinoid signaling. Phosphate esters of arachidonylethanolamine (Anandamide) is known to be prodrug of anandamide (Juntunen *et al.*, *Eur J Pharm Sci* 2003;19:37-43). In animal model, EAP accumulates in large quantities in the brain, which is correlated with the expression pattern of FAAH.

Conclusions: A plausible hypothesis is that depletion of inactive form of fatty acid amides in the brain cause depressive mood, which results in peripheral reduction of its hydrolysate compounds, *i.e.* EAP, ethanolamine and taurine. Although preliminary, our study supports the hypothesis. Further larger studies on this association are needed in order to establish link between the biomarker and endocannabinoids.

(34) DEMONSTRATING PERIPHERAL RESTRICTION OF NOVEL CANNABINOID CB1 ANTAGONIST TM38837 BY EVALUATING FOR EXPRESSION OF CONDITIONED FEAR IN MICE

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Several studies have shown that activation or blockade of cannabinoid receptor type 1 (CB1) stimulates or inhibits food intake, respectively. Rimonabant was the first selective CB1 antagonist introduced into clinical practice for obesity treatment, but due to the notably increased rates of depression, anxiety, and suicidality it was withdrawn from the market (*Le Foll et al.,* Psychopharmacology *2009; 205: 171-174*). Preclinical studies including experiments in mutant mice with cell-type specific deletion of CB1 receptors suggest that anxiety-like behavior is mediated by central CB1 receptors, while food intake and metabolism are also highly affected by the peripheral CB1 receptors (*Marsicano et al., Nature 2002; 418: 530-534; Quarta et al., Cell. Metab. 2010; 273-285*). We have evaluated a novel peripherally restricted CB1 antagonist (TM38837) in terms of fear promoting consequences of systemic vs. intracerebral injections. TM38837 has been designed to have negligible CNS penetration while fully preserved peripheral actions.

Methods: Male C57BL6/N mice underwent auditory-cued fear conditioning, followed by extinction training 1, 2, 3, and 10 days later that consisted of prolonged exposure to the auditory cue (180 s; *Plendl and Wotjak, J. Neurosci. 2010; 30: 4990-4998*). Different groups of mice were treated *per os* (p.o.) with TM38837 (10, 30 or 100 mg/kg), rimonabant (=SR141716: 10 mg/kg; a brain penetrating CB1 antagonist which served as a positive control) or vehicle, 2 h prior the extinction training on d1, d2 and d3. To assess whether TM38837 would affect fear extinction if it could by-pass the blood brain barrier, we injected TM38837 (10 or 30 µg/mouse), rimonabant (1 µg/mouse) or vehicle *intracerebroventricularly* (i.c.v.) to new groups of conditioned mice 30 min. prior the extinction training.

Results: TM38837 at the high dose (100 mg/kg; p.o.) induced a significant increase of freezing behavior as compared to vehicle-treated group, similar to that induced by rimonabant (10 mg/kg; p.o.) ($p<0.001$). At lower doses (10 and 30 mg/kg; p.o.), however, TM38837 failed to induce any significant difference in the freezing response. Both compounds (TM38837 and rimonabant; i.c.v.) caused a sustained fear response to the tone, which was more pronounced after rimonabant treatment.

Conclusions: TM38837 has been shown to be equipotent to rimonabant with regard to weight loss and effects on other metabolic parameters in rodent obesity models; hence these results are very encouraging for further development of TM38837 as a peripherally restricted CB1 receptor antagonist for indications such as obesity and metabolic disorders without inducing psychiatric side effects.

(35) EFFECTS ON FEAR AND ANXIETY OF AAV VECTOR-MEDIATED OVEREXPRESSION OF MAGL IN GLUTAMATERGIC NEURONS OF THE BASOLATERAL AMYGDALA

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In the brain, the endocannabinoid system is involved in the regulation of a large variety of functions, including the support of extinction of fear memories. The basolateral amygdala (BLA) is crucial for the modulation of emotional memory and contains cannabinoid type 1 (CB1) receptor and the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG). It was previously demonstrated that CB1 receptor-deficient mice are strongly impaired in short-term and long-term extinction in auditory fear conditioning, with unaffected memory acquisition and consolidation. To further understand the mechanisms underlying the memory extinction process, we aimed at specifically reducing 2-AG levels in glutamatergic neurons of the BLA by conditional overexpression of the 2-AG degrading enzyme monoacylglycerol lipase (MAGL). To this end, a recombinant adeno-associated virus (AAV) vector containing a short transcriptional stop cassette flanked by loxP sites in front of approximately 1 kb of the mouse MAGL cDNA was used. The stop-MAGL-AAV was administered bilaterally into the BLA of transgenic mice and wild-type littermates of the NEX-Cre line via stereotaxic delivery. In the NEX-Cre mouse line, Cre-recombinase expression is driven by the NEX regulatory sequences, driving expression exclusively in glutamatergic cells. Only in glutamatergic neurons in the transgenics (i.e. in the presence of Cre-recombinase) the loxP flanked stop element is excised to induce transgenic MAGL overexpression. At four weeks post-injection, the animals were tested in elevated-plus maze, open field, light-dark test and auditory fear conditioning to assess the influence of reduced 2-AG signaling in glutamatergic BLA circuitry on the processing of innate and learned fear.

(36) AAV-MEDIATED OVEREXPRESSION OF THE CB1 RECEPTOR IN THE MPFC OF ADULT RATS ALTERS COGNITIVE AND EMOTIONAL BEHAVIOR

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The endocannabinoid (ECB) system is strongly involved in the regulation of cognitive processing and emotional behavior. Evidence indicates that cannabinoid signaling might affect cognitive abilities inter alia by modulations of prefrontal cortical functions. Aim of the present study was to examine the specific role of the CB1 receptor in the medial prefrontal cortex (mPFC) in cognitive and emotional behaviors. Therefore, the CB1 receptor was overexpressed by adeno-associated virus (AAV) vector-mediated gene transfer specifically in the mPFC of adult Wistar rats. Animals were then tested in different anxiety-related paradigms for emotional reactivity (e.g. elevated plus maze (EPM), light/dark emergence test (EMT), social interaction) and the attentional set shift task (ASST) - an adaptation of the human Wisconsin card sorting test - for cognitive abilities and behavioral flexibility. Subtle differences in anxiety-related and exploratory behavior were found in CB1 receptor overexpressing animals (CB1-R) compared to empty vector injected controls (Empty) in the EMT and EPM, although general locomotor activity did not differ between the groups. During social interaction testing, CB1-R animals were less irritated by the unknown conspecific than controls and showed more exploratory behavior towards the social partner. In the ASST, impaired reversal-learning capabilities were detected in CB1-R animals. In conclusion, upregulation of the CB1 receptor specifically in the rat mPFC induces differences in emotional reactivity and impairs behavioral flexibility. These findings might be relevant for neuropsychiatric disorders, since higher cortical CB1 receptor expression levels have been described post-mortem in schizophrenic patients.

(37) ACUTE AND CHRONIC EFFECTS OF CANNABINOIDS ON THE BEHAVIOUR OF ZEBRAFISH EMBRYO

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Cannabinoids are natural or synthetic compounds related chemically to Δ^9-tetrahydrocannabinol (Δ^9-THC), the principle psychotropic constituent of the hemp plant, Cannabis sativa L. Here, we examine the effects of cannabinoids using the zebrafish embryo model coupled with behavioural recording. This model has been proposed as an in vitro whole animal model that could bridge the gap between cell assays and rodent assays. We exposed embryos to either Δ^9-THC, or to the synthetic cannabinoids Win 55,212-2 and CP 55,940. Over 1,700 wild-type zebrafish embryos, including controls, were cultured individually in defined buffer in 96-well plates. Geometric range-finding was used to determine the experimental concentrations. In an acute exposure regime, embryos were exposed for 1-12 h. For chronic exposure, embryos were treated for 96 h. LC50 was determined based on mortality at 5 days post fertilisation. At this time point, behavioural analysis (visual motor response test) was carried out and total distance moved was recorded. With chronic exposure, embryos habituated to the effects of all three cannabinoids (dark challenge phase). Equally, in the acute exposure regimes, we found that all three compounds produced excitement followed by suppression of locomotor activity. This acute exposure response resembles behavioural effects reported for adult rodents. Furthermore, in acute regimes, we also exposed embryos with CB1 antagonist AM 251 (AM). AM 251 attenuated the excitement produced by all three compounds. We examined the embryos for a range of malformations after cannabinoids exposure. Only Δ^9-THC was associated with a significant increase in malformations (yolk sac and pericardial oedema, and bent tail). We conclude that zebrafish are a potentially useful model for studying and screening of psychotropic compounds.

Methods: Zebrafish embryos were chronically (Administrations of cannabinoids for 96 h) and acutely (Administrations of cannabinoids for 0-12 h) exposed to Δ^9-THC, WIN 55,212-2 and CP 55,940. Mortality rate was recorded at 48, 72, 96 and 120 hpf in both range-finding and test concentration experiments, by examination under a dissecting stereomicroscope. At 5 dpf, the live embryos were subjected to the visual motor response test or light-dark-light challenge test (Champagne, et al., 2010; Steenbergen, et al., 2010; Shaukat Ali, et al., 2011a).

Results: 1) In chronic exposure regime, all three compounds showed habituation in the challenge phase of visual motor response test or light-dark-light challenge test. **2)** In acute exposure regime, all three compounds showed biphasic (stimulation at low doses and suppression at high doses) locomotory response. **3)** AM 251 attenuated the stimulation caused by all three compounds.

Conclusions: Our results have demonstrated that **a)** there are significant similarities between the behavior pattern of zebrafish embryos and rodents after exposure to selected cannabinoids. **b)** CB1 receptor is probably playing some role in regulating the excitement produced by CB1 agonists. **c)** This suggests that cannabinoid response pathways of zebrafish embryos might share significant similarities with those of mammals. **d)** It also suggests that zebrafish embryos could be a useful tool for the preclinical screening of at least some types of psychotropic drug. **e)** The presence of CB receptors in zebrafish embryos, and their strong affinities with different cannabinoids, could make zebrafish embryos useful for understanding the pharmacological properties of natural, synthetic and endogenous cannabinoids.

(38) THE IMPACT OF THE ENDOCANNABINOID SYSTEM ON SOUND LOCALISATION

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During sound source localisation animals exploit the differences in arrival time and amplitude of sound waves at the two ears. These cues are computed in the auditory brainstem in the medial superior olive (MSO) and the lateral superior olive (LSO), respectively. An exact tuning and a high temporal precision of these auditory neurones are required to allow for the accurate detection of sound sources. Despite the required precision, dynamic changes induced by neuromodulators have recently started to emerge in this system.
In other regions of the auditory brainstem a role of endocannabinoids as neuromodulators has already been described. Therefore it is plausible that the endocannabinoid system plays a role in adjusting the temporal fine tuning of MSO and LSO neurones by pre- and postsynaptic mechanisms.
Methods: We investigated the role of the endocannabinoid system in the MSO and LSO using immunohistochemical stainings and *in vitro* patch-clamp recordings from visually identified neurones in acute brain slices. As an experimental animal the Mongolian gerbil (*Meriones unguiculatus*) was used, because these animals are especially suited model organisms for auditory research, as they hear the same frequency spectrum as humans and have a similar organisation of the auditory circuitries.
Results: Immunohistochemical stainings indicate an abundant expression of the cannabinoid receptor CB1 in the MSO and LSO. In young gerbils ranging from P10 – 15 we found a predominant presynaptic localisation of CB1. This distribution completely reverted during late postnatal development and from around P20 onwards almost exclusive postsynaptic and somatic localisation of CB1 was observed. The endocannabinoid-synthesising enzymes Diacylglycerol lipase α/β were localised to the soma of postsynaptic cells at all developmental stages tested.
In line with these results, in P10-P15 gerbils depolarisation-induced suppression of inhibition (DSI) and depolarisation-induced suppression of excitation (DSE) - judged by synaptic currents - were successfully elicited. In animals older than P20 electrophysiological evidence for presynaptically located CB1 receptors could not be found, however a modulation by endocannabinoids of glycinergic currents was observed. In addition, current clamp recordings suggest an increase of K^+ currents caused by CB1-dependent signalling.
Conclusions: These results suggest that the endocannabinoid system plays an important role in the physiology of auditory neurones. In animals aged between P10-P15 over-excitation of these neurones might suppress those inputs equipped with CB1 receptors in a retrograde manner, whereas in older animals endocannabinoids seem to adjust the temporal tuning of these neurones by modulating K^+ currents and glycinergic currents.

(39) CANNABINOID POTENTIATION OF GLYCINE RECEPTORS CONTRIBUTES TO CANNABIS-INDUCED ANALGESIA.

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Recent studies have shown that psychoactive and nonpsychoactive cannabinoids can increase the activity of native and recombinant glycine ion channel receptors (GlyRs). However, little is known about the mechanisms and behavioral implication of cannabinoid potentiation of GlyRs. To address these questions, we conducted following experiments using various approaches involving electrophysiological recording, mutagenesis analysis, NMR spectroscopic analysis of the purified transmembrane domain of a GlyR subunit, chemical modification of THC, radioligand binding and several pain sensation tests in mice. Cannabinoids enhance the function of Glycine receptors (GlyRs). We have identified a serine (S) at 296 in the GlyR protein critical for the Δ^9-tetrahydrocannabinol (THC), a major psychoactive component of marijuana, -induced potentiation of I_{Gly}. The polarity of the amino acid residue at 296 and the hydroxyl groups of THC are critical for THC potentiation, suggesting a hydrogen bonding interaction between THC and GlyRs. Consistent with this hypothesis, a chemically modified THC with substantially reduced binding affinity to CB1 receptors remained equally potent in potentiating I_{Gly}. Removal of the hydroxyl groups of THC results in a compound that does not affect I_{Gly} when applied alone but selectively antagonizes cannabinoid-induced potentiating effect on I_{Gly} and analgesia in tail-flick test and in chronic inflammatory pain induced by CFA paw injection in mice. The cannabinoid-induced analgesia is absent in mice lacking a3GlyRs, but not in those lacking CB1 and CB2 receptors. These findings reveal a novel mechanism underlying cannabinoid potentiation of GlyRs, which could contribute to some of the cannabis-induced analgesic and therapeutic effects.

(40) THE ROLE OF CB2 RECEPTORS IN MODULATION OF INFLAMMATORY PAIN

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Inflammation can lead to a sensitization of peripheral nociceptors or can sensitize the spinal neurons causing hypersensitivity and spontaneous pain. Recently it was shown that CB_2 receptor agonists have analgesic effect in a tissue injury model of persistent pain. In this study we investigated the role of CB_2 receptors in the development of inflammatory pain.

Methods: We examined the nociceptive reaction of CB_2 receptor knockout and wild type animals using formalin-induced inflammatory pain. Furthermore, we studied the analgesic effect of a natural CB_2 agonist, E-β-caryophyllene ((E)-BCP), in formalin-induced inflammatory pain. (E)-BCP is a newly identified CB_2 receptor agonist that is found in the essential oils of different spices and food plants. JWH-133, a highly selective CB_2 receptor agonist, was used as reference compound.

Results: Wild type and CB_2 knockout animals showed the same pain reactions in the early and late phase of the formalin test. Both strains presented a thermal hyperalgesia one hour after formalin treatment. Orally administered (E)-BCP caused a significant analgesic effect in the late phase of the formalin test in wild type animals, but had no effect in mice lacking CB_2 receptors. In comparison to JWH-133, (E)-BCP was more effective in this inflammatory pain model.

Conclusion: Our results suggest that CB_2 receptors do not play a direct role in the development of formalin-induced inflammatory pain. However, CB_2 receptor agonists, (E)-BCP and JWH-133, are potential analgesic substances for the treatment of inflammatory pain.

(41) TARGETING NEUROPATHIC PAIN THROUGH ENDOCANNABINOID RECEPTOR SIGNALING

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Introduction Neuropathic pain is associated with inflammation or injury of peripheral and central nerves. Current pharmacotherapies either lead to inadequate pain relief or psychoactive side effects. Recently selective agonists targeting the cannabinoid receptor 2 (CB_2) came into focus for the treatment of neuropathic pain. They appear to be antinociceptive in animal models of neuropathic pain without unwanted central side effects. E-β-caryophyllene ((E)-BCP) was now found to be a natural CB_2 selective agonist, occurring in spice and food plants.
Objectives In this study we want to investigate the efficacy of (E)-BCP to attenuate neuropathic pain symptoms in mice.
Methods A partial ligation of the right sciatic nerve was performed in male wild type and CB_2 receptor knockout mice to induce neuropathic pain. Different doses (0.1, 1, 5 and 10 mg/kg) of (E)-BCP were orally administered. Thermal hyperalgesia and mechanical allodynia were assessed at day 3, 6, 8, 10 and 14 after ligation. Another selective CB_2 agonist, JWH-133 (1mg/kg), was used as reference compound.
Results Oral administration of (E)-BCP attenuated neuropathic pain symptoms (hyperalgesia and allodynia) in the behavioral readouts compared to vehicle treated mice. As expected, it did not influence the behavioral responses of CB_2 receptor knockout mice. Compared to JWH-133, (E)-BCP treatment was more effective in this model.
Conclusion The presented data show that (E)-BCP is a potential analgesic substance for the treatment of neuropathic pain with high efficacy at low dose. As it is found in different spice and food plants, a daily intake with vegetable food could be an efficient modulator of persistent pain states.

(42) CB2 RECEPTOR MEDIATES PHAGOCYTOSIS AND SCAR FORMATION AFTER MYOCARDIAL INFARCTION IN MURINE HEART

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Introduction: Reperfusion of a myocardial infarction induces transient inflammatory response and tissue remodelling with scar formation. We showed macrophage infiltration-dependent formation of granulation tissue and rapid scar development in mice. Recently, endocannabinoid receptor CB2 has been associated with modulation of macrophage function and therefore we investigated its role in murine myocardial infarction.
Methods: One hour LAD-occlusion was followed by reperfusion over 6 hrs, 1, 3 and 7 days in C57/Bl6 (WT)- and $CB2^{-/-}$-mice (n=8/group). Hearts were processed for functional, morphological, and mRNA-studies, and macrophage cell culture was used for studies of underlying mechanisms.
Results: We found transient CB2 mRNA-expression in reperfused infarction in WT mice, concomitant to a transient macrophage infiltration and rapid phagocytosis of dead cardiomyocytes with formation of granulation tissue after 3 days reperfusion. Myofibroblast differentiation followed thereafter leading to a compacted collagen deposition and completed scar formation after 7 days. In contrast, CB2-deficiency led to a prolonged phagocytosis of dead cardiomyocytes resulting in delayed granulation tissue formation. Macrophage infiltration was comparable between the strains, but in contrast to WT mice, $CB2^{-/-}$-mice showed myofibroblast persistence and less compacted collagen in the scar after 7 days reperfusion. The catheter-based left ventricular function measurements showed significantly worse ejection fraction in $CB2^{-/-}$-mice (37.2±6.5 % vs. 17.0±1,7 %, $p<0.05$). The mRNA-analysis showed significantly lower induction of TNF-α, CCL2 and CCL4 in $CB2^{-/-}$-mice, while anti-inflammatory IL-10 and remodelling related TGF-b1 and tenascin C were not induced in $CB2^{-/-}$-hearts. The macrophage cell culture showed imbalanced mRNA induction of TNF-α without subsequent IL-10 response upon IFN-g stimulation.
Conclusion: Endocannabinoid receptor CB2 seems to regulate expression of inflammatory mediators directly affecting macrophage phagocytosis activity and myofibroblast function in reperfused infarction with significant impact on myocardial scar formation and function.

(43) EFFECTS OF HIGH GLUCOSE AND INSULIN ON VASORELAXATION TO ANANDAMIDE

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Endocannabinoids, acting via central and peripheral cannabinoid receptors, are involved in the control of food intake and energy balance. Of note, the metabolic parameters, high glucose and insulin have been shown to modulate the actions and tissue content of anandamide (an endocannabinoid) in pancreatic beta-cells, adipoctyes and serum (1-2). Interestingly, anandamide is also a potent vasorelaxant and has been implicated in blood pressure regulation (3). However, the influence of glucose and insulin on the vascular effects of anandamide remains undetermined. In this study, small mesenteric artery and aorta were isolated from male Wistar rats (200-350g, killed by cervical dislocation) and maintained at 37^{o}C in oxygenated Krebs-Henseleit solution (with 10mM glucose) for isomertric tension recording. Vessels were precontracted with 10μM methoxamine (an α_1-adrenoceptor agonist), followed by cumulative additions of anandamide. Data are expressed as mean±s.e.m (n≥4rats) and analysed by Student's *t*-tests or 2-way analysis of variance. In the aorta, high glucose (30mM for 1h) significantly reduced relaxation to anandamide (relaxation at 10μM, 10mM glucose = 40±4%; at 30mM glucose = 17±9%; $P<0.05$). Response to 10μM anandamide was also reduced in the presence of 0.1μM insulin (at 10mM glucose, 16±10%; $P<0.05$). In contrast, mesenteric relaxation to anandamide was not affected by high glucose (control: pEC_{50} = 6.97±0.17, R_{max} = 106±9%; 30mM glucose: pEC_{50} = 6.78±0.17, R_{max} = 104±6%). Insulin (0.1μM) also had no effect (at 10mM glucose, control: pEC_{50} = 6.71±0.09, 100±5%; + insulin: pEC_{50} = 6.70±0.15, R_{max} = 109±12%). High glucose also significantly reduced the methoxamine-precontracted tone in aorta (by 39±10%), but not mesenteric artery. To conclude, high glucose and insulin reduce vasorelaxation to anandamide and vasocontraction to methoxamine, depending on the vascular region. The compromised anandamide response might contribute to vascular changes seen in hyperglycaemia and/or hyperinsulinaemia.

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(44) THE ENDOCANNABINOID ANANDAMIDE MEDIATES HYPOXIC PULMONARY VASOCONSTRICTION VIA FATTY ACID AMIDE HYDROLASE (FAAH) METABOLIZATION PRODUCTS

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Introduction: Anandamide (AEA) was identified as endogenous cannabinoid receptor ligand in the brain but it also has important functions in the vasculature. While the effect of AEA on vascular tone in systemic arteries has been extensively studied, its role in the pulmonary vasculature is still under debate.
Methods: In order to determine the effect of AEA on the tone of small pre-capillary resistance arteries of the lung we applied the isolated perfused lung (IPL) model of mouse under normoxic and hypoxic ventilation. The pathophysiological relevance of our findings was proven in chronic pulmonary arterial hypertension generated in hypoxia chambers.
Results: In the IPL system AEA induced a strong pulmonary vasoconstriction which was independent from CB1 and CB2 receptors but could be blocked by the fatty acid amide hydrolase (FAAH) inhibitor URB-597. Similarly, AEA was without effect in FAAH KO mice. Vasoconstriction by AEA was mediated via arachidonic acid (AA), cyclooxygenase and lipoxygenase pathways. Moreover, the cysteinyl leukotriene receptor-1 antagonist montelukast, which is approved for the treatment of asthma, could diminish vasoconstriction by AEA. Importantly, hypoxia increased AEA and AA levels in the lung and hypoxic vasoconstriction was reduced after inhibition of FAAH by URB-597 or in FAAH KO mice. Finally, also the development of chronic pulmonary arterial hypertension by hypoxia was inhibited in FAAH KO animals.
Conclusion: These data indicate that FAAH-dependent AEA degradation products are capable of inducing vasoconstriction in the lung and this pathway may be a novel and important mechanism of hypoxic pulmonary vasoconstriction.

(45) STRATEGIC LOCALIZATION OF TRPV1 EXPRESSING CELLS IN THE BRAIN VASCULAR SYSTEM

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Introduction: Cellular processes in neural death and protection in ischemic brain injury are of great importance and much effort has been dedicated. Numerous studies showed that endocannabinoids are involved in ischemic brain injury. However, results are conflicting to support either protective or harmful role of endocannabinoids in ischemia, which may be due to the complexity of the system: There are numerous receptors for endocannabinoids and their metabolites, activated by multiple ligands, inducing different biological effects. Among these receptors, transient receptor potential cation channel subfamily V member 1 (TRPV1) is suggested to play a role in regulation of blood-brain barrier (BBB) permeability. Here we investigated the precise distribution of TRPV1 in the rat brain focusing on its expression in the vascular system and sought to find a potential role of these receptors in the pathogenesis of post-ischemic brain damage.

Methods: Brain samples from male Wistar rats were used for this study. Paraformaldehyde (4%)-perfused brains were cryo-sectioned in 25µm and immunohistochemically stained with a free-floating method using antibodies against TRPV1, cannabinoid receotor 1 (CB1) von Willebrand factor (VWF) as an endotherial marker, agrin and laminin as basal membrane markers, platelet-derived growth factor receptor β (PDGFRβ) as a pericytes marker, and glial fibrillary acidic protein (GFAP) as an astrocyte marker.

Results: TRPV1 immunopositive cells were widely distributed in the brain but the level of expression was low in neurons. While CB1+ cells were found mainly in axonal terminals, the prominent expression of TRPV1+ cells were rather on blood vessels. Strong expression of TRPV1 was found in the arachnoid mater and ependymal cells in choroid plexus and subventricular areas. Double immunofluorescent staining confirmed that the perivascular and juxtavascular TRPV1+ cells were partially PDGFRβ+ pericytes and partially GFAP+ astrocytes.

Conclusions: Here we show the perivascular, juxtavascular and subventricular localization of the TRPV1 expressing cells in the rat brain. This strategic localization is indicative for its role in regulating cerebral blood flow, BBB permeability, and neurogenesis, which might well participate to the post-ischemic cellular processes in brain, such as neural death, neuroprotection, and neurogenesis.

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(46) SINGLE DOSE ADMINISTRATION OF CANNABIDIOL AFTER BRAIN HYPOXIA-ISCHEMIA AFFORDS LONG-LASTING NEUROPROTECTION IN NEWBORN RATS

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Background: Studies carried out so far demonstrated that CBD reduces hypoxic-ischemic (HI) brain damage in vivo in newborn animals in the short term.
Aim: to demonstrate that CBD neuroprotection is sustained in the long term, and is free from side effects on neurodevelopment.
Methods: unilateral HI brain damage was induced in newborn Wister rats (7-10 day-old: P7-P10) by exposure to hypoxia (10% FiO2) for 120 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups were treated s.c. with vehicle (HV, n=22) or with CBD 1 mg/kg; three schedules for CBD administration were studied: single dose (HC1D, n=23), three doses every 24 h (HC3D, n=16), or one dose every 8 h for 48 h (CBD8H, n=20). Other animals without HI served as controls, receiving vehicle (CV, n=16) or CBD (CC,n=16). On month after HI (P40) some neurobehavioral test were performed, testing contralateral paresis (Cylinder Rear Test, CRT), coordination (Rotarod, RR) and memory (Novel Object Recognition, NOR). Then rats were sacrificed, transcardially perfused with paraformaldehide (PFH) 4% and their brains removed. Then a MRI scan were carried out in PFH brains to assess the volume of lesion (VOL). Finally, a histological study by Nissl staining was carried out in brain frontoparietal cortex and CA1 area of hippocampus, scoring the tissue damage from 0 (normal) to 5 (massive destruction).
Results: HI led to a VOL equivalent to 19.6±0.5% brain volume; peri-infarct tissue appeared as damaged too (score: 0.2±0.1 vs 3.9±.3 in CA1 and 0.4±0.1 vs. 4±0.3 in cortex, for control and HV, all $p<0.05$). Brain damage was associated to permanent sequelae affecting movement of contralateral paw (ipsilateral paw preference in CRT: -1.8±4 vs. 12±4% for control and HV, $p<0.05$), coordination (permanence on RR: 248±10 vs 199±4 sec for control y HV, $p<0.05$) and memory (NOR: 66±5 vs. 46±5% for control and HV, $p<0.05$). CBD1D reduced the VOL (16.2±0.4%, $p<0.05$ vs HV) as well as peri-infarct tissue damage (score 3±0.3 in CA1 and 2.7±0.3 in cortex, all $p<0.05$ vs. HV), and restored normal functional performance (CRT: -0.7±5%; RR: 235±6 sec; NOR: 62±5%, all $p<0.05$ vs HV y NS vs control). The other CBD administration schedules (CVBD3D and CBD8H) did not offer better results than CBD1D. In control animals, treatment with CBD did not modify the survival rate, the brain volume as assessed by MRI, the histological appearance or the neurobehavioral test performance.
Conclusions: treatment with CBD single dose after a HI insult in newborn rats reduces brain tissue damage and restores neurobehavioral function in the long term. CBD administration was free from long-term side effects on neuro-development.

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(47) ENDOCANNABINOID CONTENT IN FETAL BOVINE SERA – UNEXPECTED EFFECTS ON MONONUCLEAR CELLS AND OSTEOCLASTOGENESIS

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The major endocannabinoids (ECs) *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) and other *N*-ethanolamines act as full and partial agonists at CB1, CB2, GPR55, PPAR and TRPV1 receptors to various degrees. These receptors are also expressed in immune cells like monocytes/macrophages where they regulate different cellular processes.

In this study, potentially bioactive lipids in commercially available fetal bovine sera (FBS) were quantified by GC/MS to study their potential impact for cell culture systems and experiments involving the endocannabinoid system.

Methods: The samples were analyzed by GC/Electron Ionization (EI) mass spectrometry using an Agilent 6890N GC equipped with a 30 m HP-5MS column and a 5975C MS with triple-axis detector. Specific ions were chosen for selected ion monitoring and deuterated standards used. After derivatisation with pentafluorobenzylbromide/*N*,*N*-diisopropylethylamine and dimethylisopropylsilyl imidazole the fatty acids were quantified by GC/MS. (Obata et al. 2003, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.792,131-140). Lower limits of quantification (LLOQ) on column for the measured compound were as follows: Arachidonoylethanolamide (AEA): 40 pg, 2-arachidonoylglycerol (2-AG): 2 ng, AA: 4 ng, palmitoylethanolamide (PEA): 20 pg, oleoylethanolamide (OEA): 60 pg, stearylethanolamide (SEA): 60 pg and PGE2: 50 pg. Endocannabinoid high-content sera were compared to endocannabinoid low-content sera in different cellular systems, such as GM-CFS/RANKL-stimulated osteoclastogenesis from primary human PBMCs. Osteoclastogenesis assays using primary human monocytes/macrophages were performed as previously described (Schuehly et al., 2010, Chem. Biol. 18, 1-12).

Results: We found that several commercial FBS contain bioactive amounts of 2-AG (100-250 ng/ml) and potentially also PEA, but negligible amounts of AEA, SEA. Residual 2-AG in FBS activated primary macrophages and increased migration and RANKL-stimulated osteoclastogenesis. Furthermore, 2-AG high-content sera containing medium specifically up-regulated LPS-stimulated IL-6 expression in U937 cells. Polymyxin B beads selectively removed 2-AG and partially also AEA and arachidonic acid from sera but not the other *N*-ethanolamines (Wang et al. 2000, Anal. Biochem. 294, 73-82)

Conclusions: 2-AG concentrations in cell cultures may significantly modulate cellular processes. Mononuclear cells having strong CB receptor surface expression may be particularly sensitive towards 2-AG high-content FBS. Therefore, ECs in FBS should be monitored and controlled in biological experiments involving the endocannabinoid system.

(48) OSTEOCLASTOGENESIS INHIBITION BY A NOVEL CLASS OF BIPHENYL-TYPE CANNABINOID CB_2 RECEPTOR INVERSE AGONISTS

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The cannabinoid CB_2 receptor is known to modulate osteoclast function by poorly understood mechanisms. Here we report that the natural biphenyl neolignan 4'-*O*-methylhonokiol (MH) from *Magnolia grandiflora* is a CB2 receptor-selective anti-osteoclastogenic lead structure (K_i < 50 nM) and counteracts 2-AG at the level of cAMP.

Methods: Bioactivity-guided isolation led to the identification of a new CB2 receptor scaffold, which was extensively profiled on an array of receptors. Chemical derivatization of 4'-*O*-methylhonokiol resulted in a library of compounds that were subjected to CB2 receptor binding and functional assays. The most active compounds were tested on osteoclastogenesis assays, migration assays and LPS-stimulated TNF-alpha expression, using primary human CD14+ precursor cells.

Results: Intriguingly, MH and several derivatives triggered a simultaneous G_i inverse agonist response and a strong CB_2 receptor-dependent increase in intracellular calcium via the CP55,940 competitive binding site, indicating unexpected plasticity of CB2 receptor signalling. The most active inverse agonists from a library of MH derivatives inhibited osteoclastogenesis in RANK ligand-stimulated primary human macrophages. Moreover, these ligands potently inhibited the osteoclastogenic action of endocannabinoids. Our data show that CB2 receptor-mediated cAMP formation, but not intracellular calcium, is crucially involved in the regulation of osteoclastogenesis, primarily by inhibiting macrophage chemotaxis and TNF-alpha expression.

Conclusions: We describe a novel type of biphenyl CB2 receptor-selective ligand that exerts a unique mixed functional effect. Our data indicate that nM concentrations of 2-AG increase osteoclast formation via activation of migration, thus facilitating syncytium formation. Moreover, in our assays with M-CSF/RANKL stimulated primary human CD14+ cells CB2 receptor inverse agonists inhibit osteoclastogenesis and intracellular calcium transients do not play a role. MH is an easily accessible CB_2 receptor-selective scaffold that exhibits a novel type of functional heterogeneity and shows intriguing similarities to previously described *Cannabis*-derived biphenyls.

(49) DO CB_1 CANNABINOID RECEPTORS REGULATE INSULIN SIGNALLING IN RAT PRIMARY SKELETAL MUSCLE CELLS?

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Rimonabant decreases body weight in man and improves metabolic parameters in animal models of obesity. The contribution of peripheral targets, particularly skeletal muscle, for CB_1 cannabinoid receptor antagonism in these effects is, however, unclear (Eckardt et al., 2009 *Diabetologia* 52, 664; Lindborg et al. 2010 *Diabetes Obes Metab* 12, 722; Lipina et al. 2010 *Diabetes* 59, 375). The purpose of this study was, therefore, to investigate the expression and signalling of CB_1 cannabinoid receptors and the impact of CB1 activation and inhibition upon insulin signalling in rat primary skeletal muscle cells.

Skeletal muscle cells were cultured from vastus lateralis obtained from 180-200 g male Wistar rats according to the method of Blau and Webster (1981 *PNAS* 78, 5623). mRNA expression, cAMP measurement and downstream signalling pathways were examined by conventional methods.

Although expression of mRNA for CB_1 cannabinoid receptors was detected at marginal levels using Agilent one-color 44 000 gene microarray, qRT-PCR (Taqman) identified CB_1 cannabinoid receptor mRNA expression with Ct values of 26.5-27.5 in myoblasts, myotubes and skeletal muscle tissue. In cultured myotubes, forskolin (1 μM) –evoked elevation of cAMP was unaltered in the presence of ACEA (10 nM) or rimonabant (100 nM) for 10 minutes. Treatment with ACEA (10 nM) for 10 minutes increased activation of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase (~400% and ~150%, respectively); these responses which were significantly inhibited by rimonabant (100 nM). Insulin (100 nM) treatment of myotubes for 10 minutes increased the activation of AKT/protein kinase B (870%), glycogen synthase kinase 3α and β (200% and 230%, respectively), ERK1/2 (400%) and p38 MAP kinase (500%); pre-treatment with ACEA (10nM) for 24 hours failed to alter these responses. AICAR (1 mM) -stimulated AMP-activated protein kinase activity was also unaltered by ACEA.

These findings provide evidence for expression of functionally active CB_1 cannabinoid receptor in skeletal muscle. However, they fail to support previous reports suggesting an interaction between insulin and CB_1 receptor signalling in these cells. The impact of CB_1 receptor expression in skeletal muscle will be the subject of further investigation.

(50) CB_1 CANNABINOID RECEPTOR ANTAGONISM INHIBITS BALLOON-INDUCED NEOINTIMA FORMATION IN ATHEROSCLEROTIC MICE

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Balloon angioplasty is a mechanic procedure aimed to improve blood flow in a stenotic artery, typically due to atherosclerosis. The resulting arterial injury stimulates vascular smooth muscle cell proliferation and inflammation, which may result in excessive neointima formation. Increasing evidence suggests an increase of endocannabinoid levels and receptor activation in pathological processes including atherosclerosis. In a recent study, we found that CB_2 receptor activation is protective in a mouse model of balloon-induced arterial injury. However, the role of CB_1 receptors and potential changes in endocannabinoid levels in this pathogenic condition remain unclear. The objective of this study was (1) to investigate the role of endocannabinoids in balloon-induced injury and (2) evaluate the therapeutic benefit of the CB_1 receptor antagonism in this pathology.

Methods: In all experiments, we performed left common carotid balloon distension injury in weight-matched (25-30g) male apolipoprotein E-deficient (ApoE-/-) mice fed on high cholesterol (1.25%) diet for 8 weeks before the intervention. Aspirine was given a week before intervention to prevent thrombus formation. Systemic levels of FAAH metabolites anandamide, palmitoyl- and oleoylethanolamide (PEA, OEA) were determined before and after intervention. The effect of enhanced endocannabinoid levels on balloon injury was studied in high cholesterol-fed ApoE-/- mice also lacking fatty acid amide hydrolase (ApoE-/-FAAH-/-), the enzyme responsible for endocannabinoid anandamide degradation. To assess the therapeutic benefit of CB_1 antagonism, ApoE-/- littermates were randomly assigned to receive daily intraperitoneal injection of either the synthetic CB_1 antagonist AM281 (10 mg/kg) or vehicle control, with the first injection given 30 minutes before balloon injury.

Results: Systemic anandamide levels were significantly elevated in ApoE-/- mice 7 days post-balloon injury. At baseline, FAAH-deficient ApoE-/- mice had approximately 2-fold increases in systemic anandamide, PEA and OEA levels, without further increases in response to balloon dilatation. Neointima formation was significantly enhanced in ApoE-/-FAAH-/- mice as compared to ApoE-/- controls. Conversely, we found significantly reduced neointima formation in injured vessels of ApoE-/- mice treated with the CB_1 antagonist AM281. This was associated with reduced staining for the proliferation marker PCNA, alpha-smooth muscle actin and CD68-positive macrophages within dilated arteries of AM281-treated mice.

Conclusions: Our data indicate a detrimental role for endocannabinoids and CB_1 receptors in response to acute arterial injury. Selective inhibition of CB_1 receptors might offer a new therapeutic strategy for reducing restenosis in response to balloon angioplasty which merits further investigation

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(51) THE ENDOCANNABINOID SYSTEM SHOWS DIFFERENT ALTERATIONS IN *IN VITRO* AND *IN VIVO* MODELS OF HUNTINGTON'S DISEASE

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Introduction: In this investigation we analyzed the main components of the so-called "endocannabinoid system" (ECS) in R6/2 mice, a widely used model of Huntington's disease (HD).

Methods: We measured the endogenous content of anandamide (AEA) and 2-arachidonoylglycerol (2-AG), of their biosynthetic (NAPE-PLD and DAGL, respectively) and hydrolytic enzymes (FAAH and MAGL, respectively), and of their target receptors (CB_1, CB_2 and TRPV1) in the brain of wild-type and R6/2 mice of different ages. In addition, we measured FAAH activity in lymphocytes of R6/2 mice, in order to evaluate whether central ECS alterations were mirrored by peripheral cells.

Results: In 12-week-old R6/2 mice we found a reduction of NAPE-PLD and DAGL activity, and of CB binding, as well as an increase in 2-AG content when compared to wild-type littermates, without any other change in ECS elements. Our analysis was extended to HD43 cells, an inducible cellular model of HD derived from rat ST14A cells. In both induced and non-induced conditions we demonstrated a fully functional ECS, and we showed that HD43 cells replicate the decrease in FAAH activity (half of that measured in ST14A cells) already observed in human brain and lymphocytes of HD patients.

Conclusions: Overall, our data suggest that ECS is differently affected in mouse and human HD, and that HD43 cells are suitable for high throughput screening of FAAH-oriented drugs affecting HD progression.

(52) DISEASE-MODIFYING EFFECTS OF WIN55,212-2 IN A MODEL OF MULTIPLE SCLEROSIS IN MICE: ROLE OF CB_1 AND CB_2 RECEPTORS

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Multiple sclerosis (MS) is an autoimmune disease that affects the CNS and it is characterized by inflammation, demyelination, remyelination, gliosis and axonal damage, mainly occurring in the spinal cord. Cannabinoids have been proposed as promising therapeutic agents in MS given their capability to alleviate specific MS symptoms (e.g., spasticity, pain), but also given their anti-inflammatory and cytoprotective properties that may serve to reduce oligodendrocyte death and axonal damage. In our hands, the potent CB_1 and CB_2 agonist WIN55,212-2 delayed disease progression in a murine model of MS, e.g. mice treated with myelin oligodendrocyte glycoprotein that generates a progressive pattern of EAE induction. The purpose of this investigation was to further explore the mechanism(s) underlying this effect, paying emphasis in the well-demonstrated anti-inflammatory effects of this cannabinoid agonist. As expected, the administration of WIN55,512-2 (5mg/kg, i.p) reduced neurological disability and improved motor coordination of EAE mice. In addition, EAE mice showed a marked up-regulation of COX-2, inducible NOS and TNF-α in the spinal cord and the brainstem, responses that were attenuated after the treatment with WIN55,212-2. We also observed the presence of cell aggregates in the spinal cord of EAE mice that were significantly attenuated by the treatment with WIN55,212-2. Immunohistochemical analysis (with Iba-1 and Cd11b) of these aggregates indicated that they corresponded to microglia (resident macrophages) and peripheral macrophages. Lastly, experiments conducted with selective antagonists for the CB_1 (e.g. rimonabant) or CB_2 (e.g. AM630) antagonists revealed that WIN55,212-2 effects were apparently mediated by the activation of CB_1 but not CB_2 receptors. To confirm this observation, we are presently conducting experiments with selective agonists for both receptor types. In summary, the treatment of EAE mice with the cannabinoid agonist WIN55,512-2 reduced their neurological disability and the progression of the disease. This effect seems to be preferentially exerted through the activation of CB_1 receptors which would reduce the pro-inflammatory responses that are operating in the pathogenesis of this disease. However, a cooperative role of CB_2 receptors could not be ruled out.

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(53) EFFECTS OF *CANNABIS* USE ON MONOCYTE MIGRATION IN PATIENTS WITH MULTIPLE SCLEROSIS

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Cannabinoids exhibit immune-modifying effects on animal and human immune cells (T cells, B cells, Natural Killer cells and monocytes). For example, evidence indicates that the purported immunological shift by *Cannabis,* from a Th1 to a Th2 phenotype, is beneficial for autoimmune disease. Furthermore, cannabinoids modulate a variety of T cell activities, including cytokine secretion. While it is known that monocytes migrate to the central nervous system (CNS) in Multiple Sclerosis (MS), where they may contribute to lesion formation, little is known about *Cannabis* affecting this response. Current therapeutic strategies for treating MS are immunosuppressive in nature, endeavoring to restrict immune cells from entering the CNS. Using an unbiased migration assay and freshly isolated monocyte cells from blood samples, we studied the effects of *Cannabis* use in patients with MS as compared to healthy human subjects.

Methods: Blood was obtained by veni-puncture from the ante-cubital vein of human subjects (patients with MS and healthy subjects) under a protocol approved by the Human Subjects Committee at the University of Washington. All donors provided prior written informed consent to the procedure and use of the sample. Monocytes were isolated from the buffy coat using Midi Macs™ isolation cocktail kit for negative selection (indirect cell labeling) of monocytes. Monocytes were fluorescently stained and directly seeded into a modified boyden chamber assay for unbiased quantification of their migration potential toward set chemokines.

Results: **1)** Monocytes from patients with MS who use *Cannabis* have a 75% reduced response to CCL2, a chemoattractant involved in their migration toward inflamed CNS; **2)** Monocytes from all subjects (healthy controls and patients with MS) using *Cannabis* had a decreased migratory response toward a mix of plant cannabinoids (THC, CBD and CBN) and NAGly, a potent lipid chemoattractant.

Conclusions: Our data show that *Cannabis* use impairs monocyte cell migration and suggests that *Cannabis* use may reduce the number of monocytes invading the inflamed CNS. These data add to our understanding of how *Cannabis* use affects the human immune system, suggesting that *Cannabis* represents a disease modifying therapy that could provide benefits other than symptom relief for the patient with MS.

(54) CANNABIDIOL, NON-PSYCHOACTIVE CANNABINOID, AMELIORATES CLINICAL SYMPTOMS AND DECREASES MICROGLIAL ACTIVATION IN MOG-TREATED MICE

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Cannabis compounds, cannabinoids, have been shown to exert anti-inflammatory activities in certain experimental models of inflammatory CNS degenerative diseases. The main obstacle for clinical application of those materials are their psychoactive properties. We evaluated the effects of a non-psychoactive cannabinoid, cannabidiol (CBD), in myelin oligodendrocyte glycoprotein (MOG)-induced EAE murine model of multiple sclerosis (MS) and determined the mechanisms underlying these properties, specifically in microglial cells. We observed that peripherally given CBD (administered at the time of disease onset) ameliorates the clinical EAE symptoms as evaluated using behavioral and pathological scores. Histochemical analysis of spinal cords of MOG-injected EAE mice treated with CBD *vs* MOG-only treated mice revealed that CBD down regulates infiltration/proliferation (Iba-1 staining) and activation (Mac-2 staining) of macrophages and microglia into spinal cord white matter. Using the BV-2 mouse microglial cell line and lipopolysaccharide to induce inflammatory response, we were screening for intracellular mechanisms that might be involved in the CBD anti-inflammatory activity. We observed that CBD decreased the release of interleukin (IL)-1β and IL-6 proinflammatory cytokines from activated microglial cells. CBD inhibited the activation of STAT1 proinflammatory transcription factor and up-regulated the STAT3 factor, an element of homeostatic mechanism(s) inducing anti-inflammatory events. In conclusion, we observed that CBD exerts anti-inflammatory activities *in vivo* using the in EAE model of MS) as well as *in vitro* (using microglial cells). These activities may be mediated *via* STAT dependent pathways.

(55) A NOVEL 3D MODEL OF THE HUMAN CANNABINOID RECEPTOR 2 IN ITS ACTIVE STATE HELPS TO DEVELOP NEW RADIOTRACERS FOR POSITRON EMISSION TOMOGRAPHY (PET)

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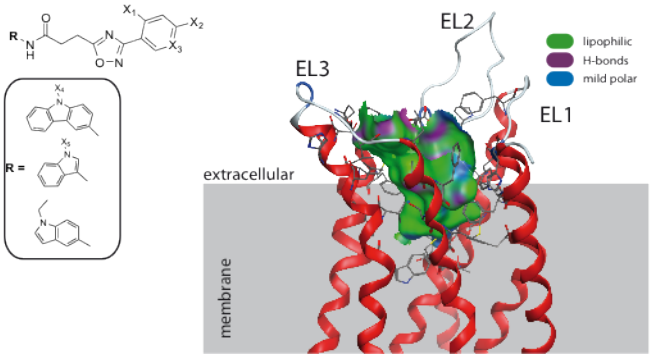
PET is a method, which provides images of functional processes in living humans. Thus, there is a strong need for highly selective positron emitting radiotracers. In order to develop such compounds for imaging of human cannabinoid receptors type 2 (hCB2) we constructed a 3 dimensional model of the human cannabinoid receptor in its active state based on the recently published X-ray structure 3qak (*Xu et al., Science 2011;332:322-327*) of the human adenosine receptor A2a (hAA2R). The model served as a guide for the rational design of hCB2-selective N-aryl oxadiazoles, which were synthetized, tested for their receptor affinity and evaluated concerning a suitable site for labeling with ^{18}F, the most preferred PET radionuclide.

Methods: The sequence of the hCB2 receptor was aligned to that of hAA2R by multiple sequence alignment. 10 models of hCB2 were constructed based on structure 3qak using the MOE software package (Molecular Operating Environment, CGC Inc., Montreal). The best model was simulated in a solvated lipid bilayer for 15 ns using the Amber03 force field as implemented in the software package Yasara (Yasara Biosciences GmbH, Wien). The N-aryl oxadiazoles were synthesized in a two-step approach. Hydroxylamine hydrochlorides and benzonitriles reacted for 24 h at 80 °C under diffuse light. After drying the intermediate, addition of succinic acid anhydrides at room temperature under diffuse light yielded the final products. K_i values of the compounds were determined in competitive radioligand displacement studies on hCB1- and hCB2-CHO cell homogenates in the presence of [^{3}H]CP55940.



Results: **1)** The 3D model of hCB2 is in good agreement with experimental data and **2)** remains in its conformation during a 15 ns MD simulation. **3)** The synthesized N-aryl oxadiazoles bind selectively on hCB2 receptors with a ratio of $K_{i(CB1)}$:$K_{i(CB2)}$ of up to 10,000. **4)** Based on molecular docking studies, the compounds most likely bind with their N-aryl-moiety (R) inside the proposed binding pocket indicating, that **5)** introduction of ^{18}F on the ring system at position 3 of the oxadiazole is most promising.

Conclusions: Our model of the hCB2-receptor provides insights into binding of ligands and allows improvement and design of hCB2-selective ligands by means of rational design. N-aryl oxadiazoles can serve as scaffolds for the synthesis of selective hCB2 PET radioligands.

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(56) ^{18}FLUORINE-LABELED NITROGEN HETEROCYCLIC DERIVATIVES AS NEW CANDIDATE FOR CANNABINOID CB_2 RECEPTOR PET IMAGING

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The type 2 cannabinoid receptor (CB_2R) is part of the endocannabinoid system and has been suggested as a mediator of several central and peripheral inflammatory disease. In normal brain, only low CB_2R expression levels were observed. However, in some pathological conditions such as amyotrophic lateral sclerosis, multiple sclerosis, stroke and Alzheimer the CB_2R is up-regulated. Peripheral CB_2R up-regulation has been demonstrated in tumors, endometrial inflammation and human and mouse atherosclerotic plaques .
In this work we report the design and synthesis of [^{18}F]-labeled naphthyridine, quinoline, and pyridine derivatives as PET tracers for *in vivo* visualization of the CB_2R as diagnostic tool for noninvasive imaging of cancer and/or neuroinflammation pathologies and for monitoring therapeutic efficacy. Furthermore the corresponding unlabeled molecules showed a remarkable affinity on CB_2R($Ki < 10$ nM).

Methods:
The synthesis procedure of some substrates was based on iodonium precursors (iodonium salts), since this approach was potentially the highest yielding in relation to the structural complexity of the studied molecules. The iodonium salts precursors of naphthyridine, quinoline, and pyridine derivatives were synthesised.
[^{18}F]fluorination experiments on suitable precursors was conducted using the microfluidic device NanoTek (Advion). Briefly, the module co-inject into a microreactor two solutions containing respectively the radiolabelling solution based on anhydrous radioactive fluoride complexed to the criptand Kriptofix® ([^{18}F]F^- K2.2.2-K^+) and the substrate that has to be fluorinated. The reaction mixtures obtained were then subjected to TLC and/or HPLC analysis to assess the yield of incorporation. Isolation of labelled compound was performed on a C-18 solid phase extraction (SPE) cartridge and was used to eliminate unreacted [^{18}F]fluoride and organic solvents (DMSO). The radiolabeled product is eluted using a small amount of ethanol, and the solution is diluted with isotonic saline and the purity of the radiolabelling compound is verified by HPLC.

Results and Conclusion:
The new procedure developed for iodonium salts synthesis avoid the need of toxic organostannanes intermediates. The employ of microfluidic synthesis techniques allowed quick optimization and performance of the labelling reaction with highly reproducibility. Moreover purification procedure on SPE were performed to obtain pure radiolabelled compound. The *in vivo* PET experiments are in progress.

(57) DEVELOPMENT OF AN ULTRA SENSITIVE LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF Δ9-TETRAHYDROCANNABINOL AND ONE OF ITS MAJOR METABOLITES Δ9-(11-OH) TETRAHYDROCANNABINOL IN HUMAN PLASMA

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Since the discovery of an endogenous cannabinoid system, research into the pharmacology and therapeutic potential of cannabinoids has steadily increased. To date Δ9 - tetrahydrocannabinol (THC) has been employed in the treatment of numerous diseases. However, more therapeutic indications for THC are currently extensively investigated. Consequently, more sensitive and specific analytical methods are needed for pharmacokinetic studies. Therefore, the objective of the present study was to develop and validate an ultra sensitive and robust LC-MS/MS method for the combined determination of THC and Δ9-(11-OH)-tetrahydrocannabinol (11-OH-THC, a major metabolite of THC) in human EDTA plasma. The analytical range for both THC and 11-OH-THC was 10.0-5000 pg/mL.

Methods: Human EDTA plasma samples, using deuterated analogs as internal standards, were subjected to a solid phase extraction step using Screening Devices C18 end capped SPE columns (3cc 100 mg), followed by a derivatization step with dansylchloride in an alkaline environment. Subsequently, the derivatized samples were subjected to a liquid/liquid extraction. Finally the samples were chromatographed on a Kinetex 2.6µ PFP 100A (100 x 3.00 mm) column. A full validation of the method was performed according to the current guidelines for bioanalytical method validation and in accordance with Good Laboratory Practice guidelines.

Results: The assay for THC and 11-OH-THC was validated in the concentration range of 10.0 - 5000 pg/mL and a Lower Limit Of Quantification (LLOQ) of 10.0 pg/mL was achieved in human EDTA plasma for both compounds. The method showed excellent accuracies and precisions for all QC levels which were determined in three validation runs and was found valid with respect to recovery, specificity, 10-fold dilution, matrix effect and stability (bench-top (24h), on machine (111h at 15°C) and freeze/thaw (three additional cycles) stability).

Conclusions: Currently, bioanalytical methods are available for the determination of THC and 11-OH-THC in human plasma with LLOQs down to 100 pg/mL. ABL has successfully developed and validated an extremely sensitive and robust bioanalytical LC-MS/MS method for the determination of THC and 11-OH-THC in human EDTA plasma with a 10-fold increased sensitivity for both compounds. The method will be applied to analyse samples from several clinical studies, including a clinical study with Namisol® (an oral tablet with THC) in patients with dementia. In this clinical phase II study the pharmacokinetic profile of THC and its metabolite 11-OH-THC and the relationship between plasma concentrations of THC and 11-OH-THC and clinical effects (behavioral disturbances) in these subjects will be determined. In conclusion, unlike other alternative methods available, this method offers an unique opportunity to investigate the pharmacokinetics of THC and 11-OH-THC in the lower plasma concentration ranges.

(58) DEVELOPMENT, VALIDATION AND USE OF A LC-MS/MS METHOD TO ASSAY FAAH ACTIVITY IN BIOLOGICAL SAMPLES

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The endocannabinoid anandamide (AEA) is hydrolyzed by the integral membrane fatty acid amide hydrolase (FAAH, EC 3.5.1.99) to arachidonic acid and ethanolamine (EA). Published assays for FAAH activity are based on the use of radioactive anandamide or alternative fluorogenic substrates. We have developed and established a novel FAAH activity assay based on LC-MS/MS measurement of tetradeutero-EA (d_4-EA) produced from tetradeutero-AEA (d_4-AEA) by the catalytical action of FAAH.

Methods: d_4-AEA and d_4-EA were quantified simultaneously by LC-MS/MS (Waters ACQUITY UPLC coupled to a XEVO TQ MS) in the positive ion electrospray ionisation (ESI+) mode after chromatographic separation on a HILIC column (ZIC-Hilic, Merck) by selected reaction monitoring (SRM). ^{13}Carbon-labelled ethanolamine ($^{13}C_2$-EA) and octadeutero-anandamide (d_8-AEA) were used as internal standards for d_4-EA and d_4-AEA, respectively. Recombinant FAAH and the FAAH inhibitor oleoyl oxazolopyridine (Oloxa) were used to determine enzyme kinetics and to find optimum conditions for substrate and protein concentration, pH, temperature and linearity. Ex vivo, FAAH activity was determined in human blood and in crude homogenates and microsomal fractions of dog liver by incubation with d_4-AEA (1 µ M) for 30 min. FAAH-catalyzed formation of d_4-EA was measured in deproteinized samples by LC-MS/MS. Total analysis time in gradient elution was 10 min.

Results: 1) Recombinant FAAH-catalyzed hydrolysis of d_4-AEA to d_4-EA was found to obey Michalis-Menten kinetics: K_M, 12.3 µM; V_{max}, 27.6 nmol min^{-1} mg^{-1} FAAH). **2)** IC_{50} of Oloxa was determined at 24.3 nM in our assay. **3)** In the microsomal fraction of dog liver, FAAH activity was optimum at protein concentrations up to 10 µg/ml and was 1.6 nmol min^{-1} mg^{-1} protein. **4)** Application of the FAAH assay and inhibitory effects of Oloxa were shown in human whole blood samples.

Conclusions: We developed a specific, sensitive and high-throughput LC-MS/MS assay for FAAH activity in biological samples such as liver and blood. This assay is useful to screen potential inhibitors and activators of FAAH activity.

(59) A NEW TLC DENSITOMETRIC METHOD IN A KIT-FORMAT FOR COMPLETE SEPARATION AND VISUALISATION OF ALL CANNABINOIDS IN CANNABIS SPECIES

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1.1 Introduction:

Cannabis simplex, cannabis preparations and cannabinoid based medicines are currently under investigation for legitimate development as medicinal agents. With the anticipation that a growing number of dispensaries who are supplying cannabis for diverse patient-groups laboratories in the private sector and the pharmaceutical industry will engage in cannabinoid analysis. Therefore, there is a need to establish a robust and cheap high-throughput screening method for routine analysis of phyto-cannabinoids in plant matrices, along with appropriate quality assurance and quality control (QA/QC). HPLC and GC, currently used in cannabinoid research, are not convenient for routine screening. Our objective was to develop and validate a cheap, standardized, non-toxic, and robust analytical tool in a kit-format for routine determination of phyto-cannabinoids in cannabis and derivatives thereof.

Methodology:

The HPTLC method developed in our laboratory (Fischedick et al, 2009) was adapted for silica G25 TLC plates and simplified for common use. The method uses 100 mg of sample matrix. It was validated with the use of pure cannabinoid reference standards. To obtain lineair calibration curves from the principle cannabinoids THC, CBD, THCV, CBG and CBD, serial dilutions (1-20%) were prepared and plated on silica G25 TLC plates. The plates were developed and colored according to the protocol. Quantification of all the spots was achieved by using the pixel counting program and the linear regression routine of photoshop/CS3 and Excell, by measuring the volume of every spot and plotted against concentration.

Results:

The method shows complete separation and visualization of all the principle cannabinoids present in cannabis samples and derivatives there of, together with all their corresponding varines. Besides the neutral cannabinoids it also reveals and visualizes the most common breakdown products CBL, CBE and CBN as discrete spots. Additionally it shows a reddish smear on the plate when the samples are cured, or aged, thus creating a new quality parameter for Cannabis. The method was shown to be comparable within a small range of error (+/- 0.5%) to results obtainable from a validated HPLC or GC method.

Conclusion:

The TLC method in portable Kit-format is reproducible and accurate for the quantification of THC, CBD, THCV, CBG and CBC, and for qualification (and even so quantification) of all the other cannabinoids. For dispensaries, the method will pave the way to introduce menu charts where on chemotype, dosage and potency indications for patients who rely on cannabis simplex for their medication.

The low-cost method can be used by people not skilled in the art, and so providing a way to introduce high-throughput screenings in quality laboratories of dispensaries and the pharmacists world wide

Fischedick JT, Glas R.J. Hazekamp A. Verpoorte R (2009) in phytochem. Anal.20: 421-426

(60) INTESTINAL *N*-ACYLETHANOLAMINE LEVELS ARE DOWN-REGULATED IN BOTH MICE AND RATS BY SENSING OF DIETARY FAT

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Introduction: There are many functions of *N*-acylethanolamines (NAEs) in the body, which beside the endocannabinoid anandamide include oleoylethanolamide (OEA) and linoleoylethanolamide (LEA). OEA and LEA are down-regulated during fasting, and intraperitoneal injections of both compounds reduce food intake in rats. This demonstrates the anorectic effect of these compounds. We have previously shown that rats fed a high-fat diet have reduced levels of NAEs in the intestine, and this reduction is independent of the energy density in the food, suggesting the presence of a fat sensor in the intestine (*Diep et al FASEB J (2011) 25(2):765-74)*, which is able to sense fat and signal a reduction of NAE levels in the small intestine. To identify the putative fat sensor, genetically modified mice (lacking specific receptors) would be suitable animal models to use. We have therefore conducted an experiment in mice, where C57bl/6 mice were fed a high-fat diet for varying days and intestinal NAE levels were determined and compared to the results from the rat studies.
Methods: All animal experiments were conducted according to the international accepted guidelines. Prior to introduction of high-fat diet, the mice were fasted for 12h, but with free access to tap water. High-fat diet (regular chow supplemented with olive oil to reach 45E% from fat) was provided and the animals were fed for either 3 or 7 days. The animals were subsequently killed and the jejunums were isolated and snap frozen. NAEs were measured as described by Artmann et al. (*Artmann et al Biochim Biophys Acta (2008)1781:200-212*).
Results: A clear time-response was seen between the duration of high-fat feeding and intestinal NAE levels in the mice. NAE levels were reduced with 31% ($p<0.05$) and 42% ($p<0.01$) after 3 and 7 days of dieting, respectively. LEA levels were after 3 and 7 days reduced with 43% ($p<0.001$) and 68% ($p<0.001$), respectively, whereas OEA levels were only significantly reduced after 7 days of dieting (24%, $p<0.05$).
In rats, total NAE levels in the intestine were reduced with 35% and 31 % ($p<0.05$ for both) after 3 and 7 days of dieting, respectively, whereas LEA levels were reduced with 44% ($p<0.01$) and 39% ($p<0.05$), respectively.
Conclusions: This study has provided us with the evidence that intestinal NAE levels in rats and mice respond in a similar manner when fed a high-fat diet. NAE levels were reduced in a time-dependent manner; the longer duration of feeding, the lower NAE levels were found the in intestine. As a result of this study, a switch from rats to mice as the experimental animal model can be conducted.

(61) CIRCADIAN RHYTHM OF BLOOD ENDOCANNABINOIDS – IS FOOD INTAKE THE KEY TRIGGER?

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Endocannabinoid signaling is an ubiquitous phenomenon throughout the vertebrate body. In the past few years, evidence has been provided for the involvement of the endocannabinoid system (ECS) in many fundamental physiological processes. Our main interest is the role of the ECS in regulating food intake and body weight. Several physiological processes follow a circadian rhythm. Evidence about ECS circadian cycles are rare, but important to reveal because disturbances in circadian rhythms have been described as important factors for the development of obesity. The purpose of this pilot study was to assess circadian changes of blood endocannabinoids in humans and to establish the role of food intake as a possible trigger.

Methods: Anandamid (AEA), 2-AG, and plasma cortisol concentrations were measured in venous blood obtained at defined time points during a standardized 24 hour daily routine (n = 4 healthy subjects). Plasma endocannabinoid concentrations were also measured in 28 subjects before, 30, 60, 90 and 120 minutes after eating a low or high fat test meal on two different days in a randomized cross-over fashion. AEA and 2-AG were determined in plasma by LC-MS/MS using deuterated internal standards. Cortisol was measured by ELISA.

Results: **1)** The 24 hour profile shows higher plasma AEA concentrations during daytime compared to values at night (0.50±0.01 vs. 0.44±0.02, $p<0.05$). 2-AG did not differ between day and night (1.85±0.88 vs. 1.63±0.70). **2)** No close correlation between endocannabinoids and plasma cortisol, which demonstrated the well known circadian rhythm, was obeserved. **3)** During the 24 hour daily cycle, AEA plasma concentrations were significantly higher before compared to 120 minutes after eating. This phenomenon was more pronounced with breakfast and lunch, and diminished at the last meal of the day. **4)** In our standardized test meal study, plasma AEA concentrations rapidly decreased after initiation of food intake, and were significantly reduced at 30, 60, 90 and 120 minutes after eating compared to baseline. **5)** There seems to be a more rapid and sustained decrease in AEA concentrations after eating a low fat meal compared to a high fat meal. However, significant differences in area under the curve did not appear ($p=0.06$). **6)** In both studies, none or less pronounced postprandial changes occurred in plasma 2-AG concentrations that never statistical significance.

Conclusions: We conclude that blood AEA, but not 2-AG, follows a circadian rhythm with lower concentrations during the night. Distinct minor circadian changes may be discovered in a larger group of subjects. However, the most prominent trigger for AEA changes during the day appears to be food intake. A rise of AEA in the fasting state in the hypothalamus, and a decrease after eating has been described in animal models. We observed a similar pattern in the blood. Additional investigations assessing the contribution of endocannabinoid signalling to circadian physiological changes are warranted.

(62) STUDIES ON THE ANORECTIC EFFECT OF *N*-ACYLPHOSPHATIDYL-ETHANOLAMINE AND PHOSPHATIDYLETHANOLAMINE IN MICE

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Introduction: *N*-acyl-phosphatidylethanolamine is a precursor phospholipid for the endocannabinoid anandamide, and other *N*-acylethanolamines such as oleoylethanolamide. It may in itself have biological functions in cell membranes. Recently, *N*-palmitoyl-phosphatidylethanolamine (NAPE) has been reported to function as an anorectic hormone (*Gillum et al., Cell. 2008; 135: 813-824*). NAPE is formed endogenously in the intestine as a response to ingested fat, and has been proposed to exert its effect in the brain. After synthesis in the intestinal cells, the compound enters systemic circulation as part of lipoproteins such as chylomicrons. In this study, two independent laboratories have investigated whether NAPE metabolites may be involved in mediating the anorectic action of NAPE *i.p.* injected in mice. Thus, the anorectic activity of a non-hydrolysable NAPE analogue, having ether bonds instead of ester bonds at *sn*1 and *sn*2 was compared with that of NAPE in molar equivalent doses. Furthermore, the anorectic effect of NAPE in NAPE-hydrolysing phospholipase D knockout mice was investigated. As negative controls, the NAPE precursor phosphatidylethanolamine (PE) and the related phospholipids phosphatidylcholine (PC) and phosphatidic acid (PA) were also tested.

Methods: Male C57BL mice were injected *i.p.* with the phospholipid compounds immediately prior to lights out. The dose was 500mg/kg or 200mg/kg NAPE, and equimolar doses of the other compounds. Food and water intake, activity and metabolic parameters were measured for 12 hours in the TSE cage system, or food intake was measured for 12 hours in the Rodent CAFE system.

Results: NAPE, the NAPE diether analogue, PE and PA all lowered 12 hour food intake and activity in mice. The effect was similar in magnitude for all four compounds. The anorectic effect of NAPE was also seen in NAPE-hydrolysing phospholipase D knockout mice. PC had no effect on food intake and locomotor activity in a dose equimolar to that of NAPE. *Post mortem* investigation revealed white flakes in the peritoneal cavity of mice treated with NAPE, ether analogue, PE and PA. No flakes were seen in the PC mice.

Conclusions: The diether analogue of NAPE and NAPE in the knockout model both lowered food intake, suggesting that the effect of NAPE is not caused by a metabolite formed *in vivo*. The controls, PE and PA were also anorectic, however. This, combined with the observation of precipitations in the peritoneal cavity of the mice, could indicate that the effect of NAPE is an unspecific effect of high dose phospholipids injected *i.p.*, which might cause malaise or pain in the animals. The lack of effect of PC could be due to a higher solubility in the peritoneal cavity of the mice.

(63) EFFECTS OF CHRONIC ADMINISTRATION OF CANNABINOID RECEPTOR INVERSE AGONIST (AM 251) ON THE FOOD INTAKE, BODY WEIGHT GAIN AND OXYGEN CONSUMPTION IN OBESE ZUCKER RATS

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Obesity is characterized by an increase in white fat mass, which results from an excess in food intake relative to energy expenditure. It is often associated with insulin resistance, dyslipidemia and hypertension, a cluster of conditions referred to as the metabolic syndrome. Several data indicates the endocannabinoids and CB1 receptors as important modulators of body weight and appetite. Based on these observations, blockade of this system has been used as an approach for the treatment of obesity and related metabolic disorders. In fact, the cannabinoid receptor inverse agonist AM 251 has been widely employed as anti obesity agent in animal models.

Methods: In all the experiments we have used presatiated lean (fa/+) and obese (fa/fa) Zucker rats (6weeks old). On group of obese rats were treated with AM 251 (3mg/kg, ip, once daily), while a second group was injected with vehicle. A third group of lean rats was injected with vehicle for 21 days. Consumption of O2 of individual animals was measured in an open-circuit indirect calorimetric system.

Results: A significant decrease in food intake throughout the experimental period was observed on the AM 251-injected when compared to the vehicle-injected obese rats. Reductions in food intake brought about by AM 251 were accompanied by significant reductions in body weight gain of these animals. A significant decrease in food intake and body weight gain was observed in vehicle-administered lean rats in comparison to obese rats injected with AM 251 and vehicle. Administration of AM 251 to dose of 3mg/kg also led to a significant increase in the consumption oxygen and the energy expenditure after 21 days of injection in comparison with vehicle-injected group.

Conclusions: The results from this study demonstrated that the cannabinoid receptor inverse agonist AM 251 activates thermogenesis, suggesting that its antiobesity property is due to the increase in energy expenditure and oxygen consumption in addition to the initial decrease in food intake. Our results represent a potentially useful approach for the treatment of obesity and metabolic disorders.

(64) METABOLIC FINGERPRINTING OF *CANNABIS SATIVA* L., CANNABINOIDS AND TERPENOIDS FOR CHEMOTAXONOMIC AND DRUG STANDARDIZATION PURPOSES

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Introduction: *Cannabis sativa* L. is an important medicinal plant. In order to develop cannabis plant material as a medicinal product quality control and clear chemotaxonomic discrimination between varieties is a necessity. In this study 11 cannabis varieties grown under controlled environmental conditions were quantitatively analyzed for terpenoid and cannabinoid content.
Methods: Cannabis plant material was grown by Bedrocan BV. Female clones of each variety were grown under the same environmental conditions. One gram of plant material was extracted 3 times with ethanol with 5 replicates per variety and treatment. Samples were analyzed by gas chromatography with both flame ionization detector and mass spectrometer. The analytical method was validated for quantitative analysis of cannabis monoterpenoids, sesquiterpenoids, and cannabinoids. Quantitative data was analyzed using principal component analysis to determine which compounds are most important in discriminating cannabis varieties.
Results: In total 36 compounds were identified and quantified in the 11 varieties. Using principal component analysis each cannabis variety could be chemically discriminated based on terpenoid and cannabinoid profile. Quantitative levels of terpenoids and cannabinoids were reproducible within varieties even with small variations in growth cycle and harvest time.
Conclusion: Our results demonstrate that terpenoids are important chemical markers in discriminating cannabis varieties when cannabinoid content is similar. The chemical profile of cannabis terpenoids and cannabinoids is reproducible within genetically identical varieties. Thus such quality controlled plant material could be used in future clinical work.

Fischedick et al., Phytochemistry 2010;71:2058-2073

(65) CANNABINOID RECEPTOR 1 IN HODGKIN LYMPHOMA AND B-CELL NON-HODGKIN LYMPHOMA ENTITIES: EXPRESSION AND FUNCTIONAL RELEVANCE FOR SURVIVAL OF TUMOR CELLS

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Cannabinoid receptor 1 (CB_1) is expressed in certain types of B-cell non-Hodgkin lymphomas (B-NHL). An analysis of CB_1 expression and function in B-cell lymphomas including Hodgkin lymphoma (HL), one of the most frequent lymphomas, was not performed to date. Here, we examined the distribution of CB_1 protein in 153 cases of HL and B-NHL. A predominant expression of CB_1 was found in Hodgkin-Reed-Sternberg cells in a vast majority of classical HL (cHL) cases while the tumor cells of nodular lymphocyte predominant subtype of HL and most of the investigated B-NHL entities were not immunoreactive for CB_1. Using lymphoma derived cell lines, we investigated the role of CB_1 signaling on cell fate. The cHL cell lines L428, L540 and KM-H2 showed strong CB_1-abundance compared to B-NHL cells. cHL cell lines displayed a dose-dependent decline of viability under CB_1 inhibition with AM251, whereas no effect was seen in B-NHL line Karpas 422. Further, application of AM251 led to decrease of constitutively active NFκB/p65, a crucial survival factor of HRS-cells, and was followed by elevation of apoptotic markers in L428 cells. Taken together, our data strongly point at CB_1 as a novel marker and a selective target for treatment of cHL.

(66) DEVELOPMENT OF CB_2 SELECTIVE CANNABINOID RECEPTOR LIGANDS AS POTENTIAL ANTITUMOR AGENTS

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Over the last few years, the interest for the cannabinoid receptor 2 (CB_2R) has been growing with proposed indications in the control of neuropathic and inflammatory pain, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, stroke, atherosclerosis, gastrointestinal inflammatory states, chronic liver diseases, and cancer. In particular, numerous pharmacological studies showed that CB_2R agonists might directly inhibit tumour growth *in vitro* and in animal models. These agents seems to discern between tumour cells and their non-transformed counterparts, therefore displaying a tumour-selectivity do not showed by common cytotoxic agents. In light of these applications, the development of potent and selective CB_2R ligands results in powerful therapeutic tools devoid of CB_1 receptor-mediated psychotropic side effects. In the present study we have synthesized new derivatives characterized by a different heterocyclic central scaffold, which were tested on CB_1 and CB_2 receptors. The newly synthesized CB_2R ligands showing the better binding profiles in the series were evaluated *in vitro* for their ability to reduce cell proliferation on a collection of tumoral cell lines.

Methods: The binding affinity (K_i values) of the compounds was evaluated in competitive binding assays against [^{3}H]CP-55,940 toward both human recombinant CB_1R and CB_2R expressed in CHO cells. The newly synthesized CB_2R ligands showing the better binding profiles, were evaluated *in vitro* for their antiproliferative activity against a large panel of human tumor-derived cell lines (MCF7, DU-145, T98G, AGS, WS-1). In particular we explored their cytotoxic effects on DU-145 cell proliferation at concentrations ranging from 0,1 to 1,0 μM after 24h. Furthermore the potential involvement of the CB_2 receptor using CB_2 antagonist was studied. Finally we analyzed the effects of the CB_2 agonists in the presence and in the absence of antagonist on the expression of the CB_2 receptor in DU-145 cells.

Results and Conclusion: The obtained results clearly suggest that the modification of the central scaffold lead to interesting effects on the CB_2R affinity. The newly synthesized CB_2R ligands are effective on different tumor cell lines and show CB2 activity as observed in DU-145 cells. In this cell line the anti-proliferative effect of the new CB_2R ligands is mediated by the CB2 receptor, indeed these compounds decrease the CB2 receptor expression levels and their effect is reverted by the CB2 antagonist.

(67) BASALIOMA TREATMENT WITH INTRATUMORAL INJECTIONS OF A CANNABIS-OIL EXTRACT: A SINGLE-CASE STUDY

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THC and other cannabinoids have been shown to exert tumour growth-inhibiting effects in many different animal models of cancer. This is achieved by inducing apoptosis, arresting cell proliferation, inhibiting angiogenesis and impairing cell invasion and spreading. Here I will present data obtained in a 92 year-old male patient whose facial basalioma was treated with a cannabis-oil extract. The treatment consisted of 6 intra-tumour injections of the extract diluted in absolute alcohol. The apparent reduction of tumour size and malignancy observed after the cannabis-treatment period encourage us to conduct more thorough studies in the future to assess whether cannabis-oil extracts may be a valid therapeutic strategy to manage skin basalioma.

(68) IMPROVEMENT OF CLONAL PROPAGATION OF *CANNABIS SATIVA* L. FOR PHARMACEUTICAL PRODUCTION

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Aeroponic propagation gives satisfaction and is efficient to observe root initiation as the cuttings remain suspended in the air. Its use is easy and the transplantation can be done in any kind of substrat. According to the use of this system 2 trials have been running on the investigation of differences in rooting ability of cutting according to its position on the stock plant and among subspecies of medicinal *Cannabis sativa.* Three cultivars with characteristic phenotype was used *indica* type, *sativa* type and hybrid (*sativa* x *indica*) type. In addition, position of the cutting on the plant stock was record, 3 different positions: top, middle, bottom. The aim of these experimentations was to improve the propagation step of the medicinal Cannabis for its production and selection in breeding program. Aeroponic propagation enable method to generate cuttings ready for transplantation in 14 days and gives clone production with fast and healthy roots growth. Difference was found between subspecies and place of cutting on plant stock. *Sativa* was found to root faster as compare with other, cuttings from bottom part of the plant stock was more suitable for clonal propagation. In taking in account these information a standardized and homogenous production of cuttings can be possible. Scientists, breeders and horticultors will have now another applicable tool to evaluate growth of roots in cuttings and a new reproducible method to obtain clones for standardized medicinal production of marijuana. Despite the satisfactory results obtained, several questions remain pending. Further research should be necessary to evaluate the impact of the stock plant age and to find out the best suitable transplantation substrat for clones obtained in aeroponic propagator according to cannabinoids production. An improvement of this clonal propagation technique can be expected and could be part of a standardized production of *Cannabis sativa* production for pharmaceutical application.

(69) CLONING OF PHARMACEUTICAL CANNABIS THROUGH AN AEROPONIC PROPAGATION SYSTEM

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Cannabis sativa L. is an important pharmaceutical species because it is the only source for a whole series of chemically diverse bioactive compounds that are currently under intensive investigation. Cuttings of pistillate plants is the preferred propagation material for the pharmaceutical production to ensure continuous chemotype correspondence of clonal progenies.
Aeroponic propagation gave satisfactory results on cloning of many economic important plant species and for that reason the aim of this study is to evaluate the feasibility of its use in the cloning phasis of Cannabis pharmaceutical production. Two experiments were conducted to evaluate the rooting capacity of three different stock plants (‘mostly sativa’, ‘sativa/indica hybrid’, ‘mostly indica’) and the rooting capacity of cuttings taken from three different positions (top, middle, bottom) of the stock plants used. Stock plants were selected from recreational strains on the basis of Δ9-THC yield per crop area unit and kept in vegetative stage in artificial growing conditions.
The aeroponic propagation system resulted easy to use and efficient to observe root initiation as the cuttings remain suspended in the air. Significant differences on rooting capacity were found between the different stock plants used and the different positions from where the cuttings were taken. The highest percentage of rooted cuttings was obtained from a ‘mostly sativa’ biotype (80%), followed by a ‘mostly indica’ biotype (70%), and the lowest value was obtained from a ‘hybrid sativa/indica’ (67%). Direct correlation was found between the percentages of rooted cuttings and the average of root lengths after 14 days from the edge of cuttings. Cuttings taken from the bottom position of stock plants had the highest rooting capacity. Cuttings were ready for transplantation in 14 days without the application of plant growth regulators. Despite the satisfactory results obtained, further research is needed to optimize the technique.

(70) ESTIMATION OF USABLE BIOMASS YIELD OF OUTDOOR CULTIVATED *CANNABIS SATIVA* L. PLANTS

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Development of Cannabis yield methodologies is essential to estimate the size of illicit cultivations and for sentencing purposes. This study was undertaken to identify factors that could predict the average yield of usable biomass under specific growing conditions. The study shows that planting density is a significant factor affecting the shape of plant canopy, which in turn affects the total plant yield. Cannabis crop was cultivated under five different planting densities i.e. 9, 18, 36, 72 and 81 square feet per plant (sq.ft/plant). At the higher planting density (9-18 sq.ft/plant), the horizontal growth rate was decreased which resulted in tall plants and narrow plant canopies, whereas, plants grown under lower planting density (36-92 sq.ft/plant) had larger branching structures coupled with increased available sunlight, soil nutrients and water which resulted in significantly greater biomass yield.

Accurate estimate of plant yield can be predicted based on the plant's fresh weight or based on the measurement of the diameter of the plant at the broadest point in the canopy. The fresh weight at the time of harvest multiplied by the number 0.1437 would result in an estimate of dry weight of useable plant material. That is, on average, 14.37% of the total wet (fresh) weight of the plant would be dry useable biomass.

A correlation was also developed between the diameter of a plant's canopy and the dry weight of the plant, resulting in an empirical formula to calculate the plant's total dry weight {dry weight = - 3.76786 + (0.06666 × diameter in cm^2)}. The percent usable biomass is then found considering that 34% of the total dry weight of non-sinsemilla plants and 58% of sinsemilla plants is usable biomass.

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(71) A QUESTIONNAIRE SURVEY OF PATIENTS PRESCRIBED SATIVEX AS UNLICENSED MEDICINE.

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Introduction
Clinical trials of the medicinal extract of cannabis, Sativex, started in 2000. Many patients have continued to use it on a "named patient" basis. We decided to determine what long-term benefits patients obtain from the use of Sativex, whether patients have changed their usage over time, and what the benefits for care-givers are.

Method
A questionnaire survey on the impact of the use of Sativex by patients who had been prescribed it as an unlicensed medicine outside of, or beyond, clinical trials, was undertaken. The Primary Objective was to identify the areas of daily function most affected by the use of Sativex. The Secondary Objectives were to study changing patterns of use, the impact on the carers and healthcare usage, and any evidence of diversion.

Results
218 questionnaires were sent out and 113 (52%) returned. 71% suffered with MS. The median daily dose was <6 sprays/day but 23% used >8sprays/day. 11% had decreased their dose and 16% increased. The main benefits were on pain (42%), spasticity 51% and sleep (5%) but 71% reported an improvement in spasticity and 82% an improvement in sleep.
The ability to lie in bed or sit in a chair, or to stand up in comfort, were the main physical benefits. 47% also had improvements in transferring. 94% said that there had been benefit to themselves in general. 30% reduced their visits to their doctor and 30% had reduced the number of accidents needing medical help.
71% of carers said that there had been benefit to the patient, and (81%) said that there had been benefit to themselves. 66% identified improvement at night. 3/113 had ever shared their medication but none had lost it.

Discussion
The impact of a medicine in clinical practice may not always be identified in the setting of a randomised double-blind controlled clinical study, which is usually designed to look at efficacy and safety.
This questionnaire survey, designed with input from the Multiple Sclerosis Trust, allowed patients and carers to describe the impact of long-term use of the cannabis based medicine, Sativex.
Patients report useful improvements across a range of activities of daily living, and sleep was a very important change. All patients were using Sativex as an add-on medicine because of inadequacy or failure of conventional therapy. Many also had other significant neurological problems thereby limiting the improvements in physical function.
The reduction in the number of accidents requiring medical attention and the reduction in the burden of care for the carers are important long-term and economic benefits.

(72) GLYCINE RECEPTORS, A NEW TARGET FOR MEDICAL MARIJUANA

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The widespread use of cannabis has been the topic of many previous and recent debates. While primary psychoactive ingredient in cannabis, Δ^9-tetrahydrocannabinol (THC), produces psychoactive effects through activation of CB1 receptors, many of nonpsychoactive chemical components of cannabis are found to provide therapeutic relief in alleviating chronic pain and other disorders. However, the targets other than CB1 receptors that mediate some of cannabis's therapeutic effects remain elusive. Here, we report that THC significantly potentiates I_{Gly} in spinal neurons and in HEK 293 cells expressing the a1 and a3 GlyRs. Using mutagenesis analysis and NMR spectroscopic analysis of the purified transmembrane domain of a GlyR subunit, we identify a serine (S) at 296 in the GlyR protein critical for the Δ^9-tetrahydrocannabinol (THC), a major psychoactive component of marijuana, -induced potentiation of I_{Gly}. The polarity of the amino acid residue at 296 and the hydroxyl groups of THC are critical for THC potentiation, suggesting a hydrogen bonding interaction between THC and GlyRs. Consistent with this hypothesis, a chemically modified THC with substantially reduced binding affinity to CB1 receptors remained equally potent in potentiating I_{Gly}. Removal of all hydroxyl groups of THC results in a compound that does not affect I_{Gly} when applied alone but selectively antagonizes cannabinoid-induced potentiating effect on I_{Gly} and analgesia in tail-flick test and in chronic inflammatory pain induced by CFA paw injection in mice. The cannabinoid-induced analgesia is absent in mice lacking a3GlyRs, but not in those lacking CB1 and CB2 receptors. Our study suggests that GlyRs are an important target that mediates some of the cannabis-induced analgesic and therapeutic effects.

(73) USE OF MEDICINAL CANNABIS IN THE NETHERLANDS

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The Netherlands has been one of the few countries worldwide to set up a governmental medicinal cannabis program. The program could be established due to a specific regulatory framework providing the necessary legal base. Probably it's the only country possessing such a framework. The program started in September 2003. Mandated by the Minister of Health the Office of Medicinal Cannabis (OMC) manages this program which provides pharmaceutical grade cannabis on prescription to chronically ill patients. Herbal cannabis is also available for scientific studies, for pharmaceutical development of cannabis-based medicines, and for export to other (mostly European) countries. As of 2011, four different standardized, and quality-controlled, cannabis varieties are available, cultivated by licensed grower Bedrocan BV. Over the years, there has been a clear and growing interest among the participants of the IACM conference in evaluating the progress of the Dutch program.

The Dutch Association for Legal Cannabis and its Substances for Medicinal Use (NCSM; www.ncsm.nl) investigated the use of prescribed cannabis over the period 2003-2010. Detailed data were provided by the Dutch Foundation for Pharmaceutical Statistics (SFK), which collects exhaustive data about the use of prescribed pharmaceuticals in the Netherlands. The SFK database covers about 90-92% of all pharmaceutical dispensations in the Netherlands. Additional data were provided by a Dutch pharmacy specialized in bulk-dispensation of medicinal cannabis. The study was performed in cooperation with the University of Utrecht, department of Pharmaco-epidemiology and Pharmacotherapy.
The data available for each individual dispensation included: the variety of cannabis dispensed, the amount dispensed in grams, the geographical location by zip code, the date of delivery, sex, age and co-medication. Each patient was identified by a random ID code only. The obtained results revealed that cannabis was dispensed more than 40.000 times to about 6.000 different patients over the study period 2003-2010. The number of patients using cannabis for medicinal purposes steadily increased in recent years, growing from about 850 patients in 2006, to more than 1.300 in 2010.
Further statistical analysis of the data revealed, among other results, the average use of medicinal cannabis per patient, the preferred cannabis varieties, geographical distribution of users and the possible indication. About half of the patients tried medicinal cannabis on prescription only once. Although this is not uncommon for drugs used by chronic patients, detailed analysis may provide further clues to factors involved in successful treatment with cannabis. About a quarter of all patients received cannabis more than 5 times, and could be considered as long-term users. In our presentation, we will analyze a potential correlation between cannabis use, illness, and co-medication used.

(74) MEDICAL CANNABIS USE IN POST-TRAUMATIC STRESS DISORDER: A NATURALISTIC OBSERVATIONAL STUDY

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Introduction: Posttraumatic stress disorder (PTSD) is a pervasive and devastating anxiety disorder, the lifetime prevalence of which, as assessed in several community-based studies, is reach up to 8%. Frequently, PTSD is associated with other mental and somatic conditions such as depression (in up to 70% cases) and severe pain of various origins. Many PTSD patients frequently use marijuana as "a emotional regulation strategy to reduce or to manage perceived aversive psychological or mood states". Meanwhile, the optimal treatment for PTSD and its comorbid conditions is still in development and the effectiveness and safety of Cannabis (Medical marijuana) use in such patients is not clear.
Methods: As a part of our routine consulting work, we assessed the mental condition of 79 adult PTSD patients, who applied to the Ministry of Health in order to obtain a license for the Medical Cannabis. The group consisted of patients with "pure" PTSD (18 patients), PTSD patients with clinical depression (27 patients) and patients suffering from PTSD/chronic pain comorbidity (34 patients). Clinician-Administered PTSD scale (CAPS) was used for traumatic symptoms assessment and Quality of Life Scale was filled out. The changes in Clinical Global Impression-Improvement scale (CGI-I) were registered. The data on their somatic conditions and pain level was provided by their treating physicians. Only part of them (about 50%) got Medical Cannabis licenses (study group). We followed up them (periodical evaluation) for a period of about two years.
Results:, Majority of PTSD patients used also the conventional medications (such as antidepressants and sedatives, pain killers etc), prescribed them by their treating physicians. Medical Cannabis (as *sativa* and/or *indica* species) was provided by several companies. The Cannabis daily dosage was in range 2-3 gr/day (containing about 20% of cannabioids active compounds THC/CBD). In most cases a significant improvement in Quality of Life and pain scores, with some positive changes in CAPS scores was observed. Under this combine (Cannabis + conventional medications) treatment, the patients reported a discontinuation or lowering the dosage of pain killers and sedative pharmacological agents. The majority of improved PTSD patients belonged to groups with either pain and/or depression comorbidity. No exacerbations or serious adverse events were reported.
Conclusion: This naturalistic observational study represents a first attempt to assess and to monitor the effectiveness and safety of the Medical Cannabis use in PTSD patients. The results show good tolerability and other benefits (especially in the quality of life & on CGI-I) of such flexible combine approach, particularly, in the patients with either pain and/or depression comorbidity. Further large-scale investigations are needed to substantiate our observations and to elaborate the most effective and safe therapeutic approaches to these difficult-to-treat group.

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(75) PSYCHOSOCIAL STRESS MODULATES CIRCULATING ANANDAMIDE AND OTHER N-ACYLETHANOLAMINES IN HEALTHY HUMANS

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Introduction: Stress plays an important role in psychiatric disorders, and recent preclinical evidence indicates that the endocannabinoid system is involved in responses to stress. Stress activates the central endocannabinoid system, which, in turn, is thought to negatively modulate HPA-axis activation during stress-recovery. This study aimed to investigate of the effect of acute psychological stress on circulating levels of endocannabinoids (eCB) and their structural analogues in healthy human volunteers.

Methods: 71 young adults participated in two sessions in which they were exposed to either a standardized psychosocial stress procedure (Trier Social Stress Test) or a control task. Blood samples for eCB and cortisol assays, cardiovascular and subjective measures were obtained before and at regular intervals after the stress or the control task. Serum levels of the endocannabinoids arachidonylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), as well as of the N-acylethanolamides (NEAs) N-palmytoilethanolamine (PEA) and N-oleoylethanolamine (OEA) were determined using isotope-dilution liquid chromatography/mass spectrometry.

Results: Compared to the control condition, stress increased serum concentrations of AEA and the other NAEs immediately after the stress period. There were no changes in 2-AG at this time. Increases in PEA were positively correlated with increases in serum cortisol after stress. Baseline (pre-task) levels of Anxiety were negatively correlated with baseline concentrations of AEA and OEA. Subject's sex and menstrual cycle status affected the NAE responses to stress. Subjects of Asian and African American ethnicity exhibited different patterns of stress responses, including differences in NAEs, compared to Caucasian subjects.

Conclusions: These results represent the first data in healthy humans indicating that stress increases circulating levels of NAEs including AEA in serum. This finding is consistent with preclinical findings supporting a role for eCBs in stress. Further research is needed to elucidate the function of these lipid mediators, and to explore their potential as therapeutic agents.

(76) THE EFFECT OF CANNABIS INTOXICATION ON EGO EQUILIBRIUM: INVESTIGATING THE ACUTE DEVELOPMENTAL IMPACT OF AN ALTERED STATE OF CONSCIOUSNESS

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Introduction: This quasi-experimental study investigated the effect of cannabis intoxication on the ego equilibrium of recreational cannabis users ($N = 30$) in an attempt to coordinate the acute altered state phenomenology with observable stages in ego development. Ego equilibrium is defined as the predominant stages inhabited within the multistage constellation of ego functioning made observable by the Washington University Sentence Completion Test (WUSCT). This research was a landmark attempt at corroborating altered state phenomenology, in its acute form, with developmental structuralism.
Methods: Recreational cannabis users with a minimum of 50 reported prior uses of the substance were recruited to self-administer cannabis through direct inhalation. Participants completed the WUSCT for ego development twice: once at baseline and a second time after cannabis intoxication was achieved.
Results: Results indicated a statistically significant increase in WUSCT summed scores from pre stage assessment ($M = 160.83$, $SD = 19.35$) to post stage assessment [$M = 170.10$, $SD = 20.64$, $t(29) = -4.815$, $p < .0005$]. The eta squared statistic (.44) indicated a large effect size, with substantial differences in before and after scores. There was sufficient evidence to indicate a statistically significant difference in pre and post stage responses as relates to the summed score derived from the WUSCT. A nonparametric Wilcoxon signed ranks test was utilized to assess the pre and post stage Total Protocol Ratings (TPR). While the assessment inherently depicts a multistage constellation of ego functioning, the TPR represents a single-stage 'average' of the multistage frequency spread. The Z-value for the Wilcoxon signed ranks test demonstrated statistical significance ($Z = -3.116$, $p = .002$), indicating that the TPR post stage ratings ($M = 4.83$, $SD = 1.21$) were significantly higher than the pre stage TPR ratings ($M = 4.37$, $SD = 1.16$).
Conclusion: Results demonstrated a statistically significant increase from pre to post intoxication responses as relates to both the summed scores and TPRs derived from the WUSCT. The majority of the sample responded from moderately to significantly higher stages in psychological development when intoxicated than when sober. Findings suggest that cannabis intoxication affected ego equilibrium and subsequent functioning in a manner that temporarily enhanced the ego's developmental constituents.

(77) THE SOCIAL AND BIOETHICAL ASPECTS OF USE OF MEDICINAL CANNABIS IN CROATIA

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Introduction: Cannabis sativa L. – hemp plant is the plant which is more and more present in public. Because of too much controversial, unsubstantiated and unexplored information, it is needed to observe this matter from its social and bioethical aspect and raise public awareness on this topic in Croatia and elsewhere in the world. Somewhere in the future hemp will become economic mainstream in the world, because it applies to many industries such as: textile, cosmetics, construction, paper, food and medical and pharmaceutical industries and as a biodegradable material. Hemp is a plant that has a wide range of applications that makes it special compared to other botanical cultures.

Materials and methods: Hemp is explored in this paper by reviewing relevant literature, direct experience during and internship at Bedrocan B.V. in the Netherlands, producer of pharmaceutical grade cannabis contracted by the Dutch government and informal interviews with respondents originating from the area near the mountain of Kalnik in Croatia, where they used to grow hemp for their own needs. In the Republic of Croatia, hemp is treated primarily as an illegal drug. It is very difficult to find research data in Croatia due to the fact that Croatian National Institute of Statistics has no information about the production of hemp.

During an internship at Bedrocan B.V. in the Netherlands I was encountered with numerous cultural, social and bioethical differences in the treatment of this plant compared to Croatia.

To be more specific: the Dutch government supports the cultivation of plans for medicinal purposes, and the Dutch public is, unlike the Croatian public, very sensitizes to a wide range of use and great utility of this plant. Hemp is a plant about which people in The Netherlands talk with less prejudice then in Croatia.

Conclusion: Medicinal use of cannabis in Croatia is very viable option because this plant has a long presence in this region in all aspects of our lives because of its usefulness in general, and the widespread traditional use as a medicine. Furthermore Bedrocan B.V. in The Netherlands is an example of good practice in the production of cannabis for medicinal purposes. Production, distribution and use of cannabis are taking place under strictly controlled conditions overseen by the Central Office (Agency) for medicinal cannabis. Patients have the option of using medicinal cannabis through the pharmaceutical system in The Netherlands, and thus improve their health.

(78) EVALUATION OF A NEW PLATFORM TO ADMINISTER CANNABINOIDS BY INHALING: MINIVAP® PORTABLE VAPORIZER

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THC and othe cannabinoids can be administed in different ways like infusion, food, sublingual spray, smoked or vaporized. It is known that inhalation is very effective because of the fast availability of the active principles and the activation of the cannabinoid acids by the process of heating (THCA to THC, CBDA to CBD). The administration of cannabis with vaporizer avoids the by-products of smoking and provides a safety delivery to the patient. This poster show the MINIVAP portable vaporizer, as a new platform to administrater cannabis in an effective, small sized and secured device. The first tests made in the University of Leiden with medical grade cannabis from Bedrocan BV, demonstrate that the process of extracting cannabinoids using the MINIVAP can be effective and reproducible for medical use. Also the information obtained from the simulations of the boiling process with a computer model of this vaporizer, shows the efficiency of the device to evaporate different susbtances. These results encourage us to continue studding and improving the device for the therapeutic administration of cannabinoids.

(79) INTOXICATIONS FOLLOWING RECREATIONAL USE OF HERBAL PRODUCTS CONTAINING SYNTHETIC CANNABINOIDS

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Introduction. Products from *Cannabis sativa* have long been used by humans for medical and recreational purposes. The psychoactive ingredient in these products is mostly Δ^9-tetrahydrocannabinol (D^9-THC), and the primary targets of D^9-THC in the brain are the G-protein coupled CB_1 cannabinod receptors. The recreational use of D^9-THC and products from *Cannabis sativa* is prohibited in most countries. In order to circumvent this prohibition, synthetic cannabinods were recently introduced to the drug market: harmless herbal mixtures for smoking are "enriched" with such synthetic cannabinoids. The information on the toxicity of these synthetic cannabinoids is very limited. Here we present a series of intoxications following consumption of herbal mixtures containing synthetic cannabinoids.

Methods. Twenty nine patients were included in this retrospective study: they were hospitalized after inhalation of herbal mixtures, and the presence of synthetic cannabinoids in their blood was analytically verified. After extraction from the serum, the synthetic cannabinoids were separated and quantified by high performance liquid chromatography and electrospray ionization tandem mass spectrometry.

Results. The patients consumed herbal mixtures with names like "Spice", "Smoke", "Bonzai" and "Maya". The following synthetic cannabinoids were identified in the serum of patients: JWH-015 (1 patient), JWH-018 (8 patients), JWH-073 (1 patient), JWH-081 (7 patients), JWH-122 (11 patients), JWH-210 (12 patients), JWH-250 (4 patients) and AM 694 (1 patient). According to the three-level (minor, moderate and severe intoxication) "Poisoning Severity Score (PSS)" (Persson HE, Clin Toxicol 36: 205-213, 1998), 34 % of the patients had a minor intoxication and 66 % of the patients a moderate intoxication. No patient was severely intoxicated. The most frequent intoxication symptoms were the following. Nervous system: restlessness / agitation (41 % of the patients), change of perception / hallucination (38 %), anxiousness / panic attack (21 %), somnolence (17 %), sopor (17 %), disorientation (14 %) and amnesia (7 %). Cardiovascular system: tachycardia (76 %), hypertension (34 %), hypotension (7 %) and dyspnea (17 %). Gastrointesitnal system: nausea / vomiting (28 %). Blood parameters: decrease in plasma potassium concentration (28 %), elevation of creatine kinase (14 %) and elevation of plasma glucose (31 %).

Conclusions. Many of the symptoms observed in the present study are similar to those observed acutely after inhalation of *Cannabis* products (see Hollister LE, Pharmacol Rev 38: 1-20, 1986): panic attacks, somnolence, changes in perception / hallucination, tachycardia and hypotension. However, some of the symptoms seem to occur only after inhalation of synthetic cannabinoids: sopor, hypotension, nausea / vomiting, decrease in plasma potassium and increases in plasma creatine kinase and glucose. We hypothesize that these "novel" cannabinoid effects are due to strong stimulation of CB_1 cannabinod receptors in the brain by the synthetic cannabinoids. Indeed, the involved synthetic cannabioids possess much higher affinity for the CB_1 receptor than Δ^9-THC (Huffman JW, Cannabimimetic indoles, pyrroles, and indenes. In: Reggio PH [ed], The cannabinoid receptors, The Receptors, Humana / Springer, Heidelberg, pp 49-94).

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Andreas **Zimmer**	University of Bonn, Germany
Alexander **Zoerner**	Hannover Medical School, Germany

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The following young scientists have received a travel award sponsored by the International Society for Neurochemistry:

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